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Evaluating the effectiveness of a Mexican strain of *Duddingtonia flagrans* as a biological control agent against gastrointestinal nematodes in goat faeces

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Abstract

The use of *Duddingtonia flagrans* in the control of goat nematodes was investigated. Initially, the time of passage of chlamydospores through the digestive tract of goats was evaluated. Two groups of seven parasite-free kids were formed. Group A received a single dose of 3.5×10^6 *D. flagrans* chlamydospores (FTHO-8 strain) per kg of live weight. Group B did not receive any chlamydospores. Faeces were obtained from each kid daily from day 4 prior to inoculation until day 5 post-inoculation (PI) and were placed in Petri dishes containing water agar. Gastrointestinal nematode infective larvae were added to each Petri dish and incubated at 25°C for 7 days. Petri dishes were examined to detect the fungus and trapped nematodes. A second trial evaluated the effect of *D. flagrans* on the number of gastrointestinal nematode larvae harvested from goat faecal cultures in naturally infected goats. Two groups of seven goats were formed. The treated group received a single dose of 3.5×10^6 *D. flagrans* chlamydospores per kg of liveweight. The control group did not receive any chlamydospores. Faeces were obtained twice daily from each kid. Two faecal cultures were made for each kid. One was incubated for 7 days and the other for 14 days. Gastrointestinal nematode larvae were recovered from each culture and counted. Percentage of larval development reduction was determined using a ratio of larvae/eggs deposited in the control and treated groups. *Duddingtonia flagrans* survived the digestive process of goats, and maintained its predatory activity, being observed from 21 to 81 h PI (3 to 4 days). A reduction in the infective larvae population in the treated group compared to the non-treated group was observed in both incubation periods (7 days: 5.3–36.0%; 14 days: 0–52.8%, $P > 0.05$). Although a single inoculation of *D. flagrans* can induce a reduction of infective larvae collected from faeces, a different scheme of dosing may be needed to enhance the efficacy of *D. flagrans* in goats.

Introduction

Infection with gastrointestinal nematodes is one of the main limiting factors affecting grazing/browsing goat production systems in the tropics especially during the rainy season, when it can cause a severe reduction in live

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weight gain or even the death of young animals (Torres-Acosta, 1999; Chandrawathani *et al.*, 2003). The treatment of animals with anthelmintic products is a common way to control the effects of gastrointestinal nematodes in goats around the world (Hoste, 2000). Although anthelmintic treatment helps to reduce parasitic burdens, there is already evidence indicating the presence of benzimidazole and levamisole resistant nematodes in goat herds of Yucatan, Mexico (Torres-Acosta *et al.*, 2002, 2003). Alternative strategies for the control of gastrointestinal nematodes are being sought to reduce the dependence on anthelmintics. Biological control is an alternative control method based on the use of living organisms that are introduced to the environment to control a target organism by reducing its population (Thamsborg *et al.*, 1999). Previous research produced encouraging results in the control of nematodes in different hosts using the nematode-trapping fungus *Duddingtonia flagrans* (Larsen *et al.*, 1995; Faedo *et al.*, 1997; Fernández *et al.*, 1997; Thamsborg *et al.*, 1999; Larsen, 1999, 2000; Knox & Faedo, 2001). In Mexico, a native strain of *D. flagrans* (FTHO-8) isolated from sheep dung (Llerandi-Juarez & Mendoza de Gives, 1998) has been tested in both *in vitro* and *in vivo* trials to explore the possibility of controlling ruminant parasitic nematodes (Llerandi-Juarez & Mendoza de Gives, 1998; Mendoza de Gives *et al.*, 1998). Resistant spores (chlamydospores) of such species have been administered to animals either through an oral suspension of fungal material (Mendoza de Gives *et al.*, 1998) or a bio-preparation consisting of chlamydospores mixed with oat grains and molasses (Mendoza de Gives *et al.*, 2005). After passing through the ruminant gastrointestinal tract, chlamydospores are expelled with dung to the environment, germinate and colonize the faecal material. The presence of recently hatched larvae stimulates the formation of a trap by the mycelia and larvae are captured and destroyed by the fungi (Faedo *et al.*, 1997). Most research regarding the use of *D. flagrans* in the control of nematodiasis of ruminants has been focused on the control of parasitic nematodes in either cattle or sheep. However, information about the possible use of this technology in goats has recently been published (Sanyal & Mukhopadhyaya, 2002; Chauhan *et al.*, 2002; Chartier & Pors, 2003; Paraud & Chartier, 2003; Wright *et al.*, 2003; Waghorn *et al.*, 2003; Chandrawathani *et al.*, 2004; Paraud *et al.*, 2004; Terrill *et al.*, 2004). These research groups demonstrated that *D. flagrans* spores survived passage through goats and significantly reduced the number of infective larvae in faecal cultures (Paraud & Chartier, 2003; Paraud *et al.*, 2004) and on plot studies (Chartier & Pors, 2003; Wright *et al.*, 2003; Chandrawathani *et al.*, 2004). Terrill *et al.* (2004) further investigated dose titration and dose timing using a Danish strain of *D. flagrans* in goats. However, there is a lack of fundamental information on the kinetics of *D. flagrans* chlamydospore passage through the gastrointestinal tract of goats. Also, the efficacy of a single dose of *D. flagrans* across time post-inoculation has not been investigated. The aims of the present study therefore were to investigate the survival and kinetics of a Mexican strain of *D. flagrans* chlamydospores after passing through the gastrointestinal tract of goats and to determine the effectiveness of a single dose of *D. flagrans* in reducing the number of parasitic larvae in goat faeces.

Materials and methods

Two experiments were performed in the Faculty of Veterinary Medicine and Animal Science, Autonomous University of Yucatan, located in Yucatan, Mexico.

Experiment 1: dynamics of the elimination of D. flagrans *chlamydospores after passing through the gastrointestinal tract of Criollo kids*

Experimental animals

Fourteen 6-month-old female Criollo goats (average of 20 kg body weight), raised nematode free were used in this experiment. Animals were allocated into metabolism crates. Goats were fed with 500 g per animal per day of a balanced feed, which contained soybean meal, sorghum meal, wheat bran, star grass hay, sugarcane molasses, vitamins (A, D and E), salt and macro- and micro-minerals. The feed was individually supplied each morning and fresh drinking water offered *ad libitum.*

Nematode infective larvae

Faecal cultures from naturally infected adult goats were made using the Corticelli Lai technique, described by Rodriguez *et al.* (1994). After an incubation period of 7 days, larvae were recovered from cultures via the Baermann technique. Identification of infective larvae was achieved following the keys of identification by MAFF (1986) and Bowman & Lynn (1999). Larvae were washed using a 50% sucrose solution following the technique described by Llerandi-Juarez & Mendoza de Gives (1998). Larvae were rinsed and re-suspended in sterile water containing 200 IU benzylpenicillin per litre of water. The mean number of larvae in suspension was estimated by counting the number of larvae in ten 0.1 ml subsamples. Clean larvae were maintained at 4°C until their utilization. Larvae obtained for the experiment included 41% of *Haemonchus* sp., 26% of *Trichostrongylus* sp. and 33% *Oesophagostomum* sp.

Evaluating the presence of nematode-trapping fungi in experimental animals before and after D. flagrans *inoculations*

Individual faecal samples were taken from the rectum of each animal for 7 days before inoculation. After inoculation with *D. flagrans*, faecal samples were obtained from experimental animals at 0, 3, 9, 21, 27, 33, 45, 51, 57, 69, 81, 93, 105, 117 and 129 post-inoculation.

The presence of nematophagous fungi was identified from individual faecal pellets (approximately 0.5 g) from each kid. A pellet was deposited on the surface of a 6 cm water agar plate (2% agar + chloramphenicol 500 mg l^{-1}). The water agar plates were sealed with Parafilm, and incubated at room temperature for 7 days. On day 2 of incubation, 500 gastrointestinal nematode infective larvae (L3) were added to each plate to stimulate the development of nematode-trapping fungal structures (Barron, 1977). After the incubation period, the surface of each plate was scanned with a stereo-microscope (4 × magnification) to identify the typical structures of nematode-trapping fungi on two consecutive days. The taxonomic identification keys for nematode-trapping

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fungi used in this trial were those by Cooke & Godfrey (1964).

Dosing with D. flagrans *spores*

All goats received a mixture of oat grain (5 g) and sugar cane molasses (2 ml) for one week prior to the initiation of the trial. Once the goats had adapted to the supplement, two experimental groups were formed. One group ($n = 7$) received a single dose of 3.5×10^6 chlamydospores of *D. flagrans* (FTHO-8 strain, Llerandi-Juarez & Mendoza de Gives, 1998) per kg of body weight. Chlamydospores were mixed with molasses and oat grains as previously described. Another group of seven goats received only the mixture of molasses and oat grains as a placebo. The criterion for selection of this chlamydospore dose per kg of body weight was based on a previous experiment (Fernández *et al.*, 1999).

Fungal isolates

A Mexican isolate of *D. flagrans* (FTHO-8), obtained from sheep dung in Mexico (Llerandi-Juárez & Mendoza de Gives, 1998), was used in this study. The fungus was cultivated on potato dextrose agar plates (Ulloa & Hanlin, 1978). Fungi were produced at the National Centre for Disciplinary Research in Veterinary Parasitology (CENID-PAVET, INIFAP), located in Jiutepec, Morelos, Mexico. Five ml of sterile water were added to the surface of 4 to 6-week-old *D. flagrans* culture plates. The fungal mass was removed from the agar surface by scrapping with a scalpel. This material was collected into a 250 ml glass flask. The fungal suspension was vigorously shaken on a stirrer with a magnetic bullet for 6 h. The supension was forced through a fine mesh with a spoon in order to separate as much mycelia and chlamydospores as possible. Most chlamydospores were retained in the mesh. This process was repeated two or three times in order to get the best separation of chlamydospores, so that the final suspension contained much less chlamydospore chains and mycelia. Chlamydospores in the suspension were counted using a Neubauer counting chamber and the suspension collected in 50 ml centrifuge plastic tubes, which were maintained at 4°C until used.

Experiment 2: effectiveness of the Mexican strain of D. flagrans *at reducing the number of parasitic larvae in goat faeces*

Fourteen goats were selected from a group of 22 goats, naturally infected with gastrointestinal parasitic nematodes. Goats were 50 weeks old and weighed an average 25 kg. Goats were selected on a mean of at least 500 eggs per gram of faeces (epg) deposited during eight consecutive days. These goats were tested negative for the presence of nematophagous fungi in faeces, following the technique described above.

Fourteen goats were randomly allocated to two groups. Each goat was kept in an individual pen with a concrete floor and received 500 g day^{-1} of feed (same as in experiment 1). Drinking water was offered *ad libitum*. Experimental animals were trained to eat the oat/sugar cane molasses mix, which was used as a vehicle for the chlamydospore treatment as described above. *Duddingtonia flagrans* treatment was performed by giving a single dose of the *D. flagrans* FTHO-8 strain as described in experiment 1 for the treated group. The control group received the mix without chlamydospores.

Sampling and processing faecal samples

Faecal samples were obtained directly from the rectum of each goat one day before treatment and every day during the following five days. Samples were collected twice a day (0900 and 1200 h). The number of eggs per gram (epg) of faeces was determined for each kid using a modified McMaster technique (Rodríguez *et al.*, 1994). Each goat had an individual bulk faecal culture made with 5 g of faeces per goat in 3.5 cm plastic Petri dishes. Cultures were kept in closed boxes and were incubated at room temperature 25–30°C and 60–80% humidity inside a glass cabinet for 7 and 14 days. Cultures were aerated and humidified every day for 30 min. At the end of the corresponding incubation periods, faecal material from each Petri dish was placed into a Baermann apparatus. Larvae were recovered after 24 h in the funnel (Rodríguez *et al.*, 1994). Recovered larvae were maintained at 4°C until counted (larvae per gram of faeces (lpg)). The number of infective larvae belonging to the order Strongylida in the two groups was estimated as described before.

Statistical analysis

Mean faecal egg outputs (epg) of treated and control groups were compared by means of ANOVA on each day post-inoculation (days 0, 1, 2, 3, 4 post-inoculation). Individual faecal egg counts were transformed (log 10 (epg + 1)) before analysis.

The percentage development for each day post-inoculation of both incubation periods was calculated as separate total ratios using the formulae by Paraud *et al.* (2004):

$$(\text{mean lpg}^{*}/\text{mean epg}) \times 100$$

The percentage of larval development reduction was calculated using the formulae by Paraud *et al.* (2004):

$$[[(\text{mean lpg of control/mean epg of control}) - (\text{mean lpg of treated/mean epg of treated})] \times 100]/(\text{mean lpg of control/mean epg of control})$$

Data of larvae were transformed log10 (lpg +1) and were analysed by covariance analysis with the covariable being log10 (individual epg + 1) in order to account for the faecal egg deposition of each individual, which varied with time. Data analysis was performed using Systat 10.0 (SPSS Inc., Chicago, USA).

Results

Experiment 1

The kinetics of *D. flagrans* chlamydospores passage through the gastrointestinal tract of goats are shown in table 1. No evidence of *D. flagrans* or other nematode--trapping fungi was recorded in the control group. *Duddingtonia flagrans* structures and trapped larvae were found in the water-agar plates of treated goats from 21 h to 57 h post-treatment (table 1). No *D. flagrans* structures

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were found at 3 or 9 h post-inoculation. Only five goats were positive to *D. flagrans* 69 h post-treatment and only two goats were positive 81 h post-treatment. No goat was positive from 93 h post-treatment.

Experiment 2

Faecal egg outputs (epg) of strongylid nematodes in control and treated groups were similar across the experiments (tables 2 and 3). However, there was day-to--day variation in egg deposition. For the 7-day incubation period, the mean number of infective larvae in cultures from the treated group tended to be lower than that obtained in the control group ($P > 0.05$) (table 2). On the other hand, for the 14-day incubation period, the mean number of infective larvae in cultures from the treated group tended to be lower than that of the control group on days 1, 2 and 4 post inoculation ($P > 0.05$) (table 3). The percentage of larval development reduction ranged from 5.3% to 36.0% in faeces incubated for seven days and from 0% to 52.8% in faeces incubated for 14 days.

Discussion

Experiment 1

In the present study, *D. flagrans* chlamydospores of the FTHO-8 strain survived after passing through the gastrointestinal tract of goats, preserving their trapping ability and their lethal action against infective larvae of different species of gastrointestinal parasitic nematodes. The results confirm previous reports in goats (Chandrawathani *et al.*, 2003; Chartier & Pors, 2003; Paraud & Chartier, 2003; Waghorn *et al.*, 2003; Wright *et al.*, 2003; Paraud *et al.*, 2004; Terrill *et al.*, 2004). The fact that *D. flagrans* chlamydospores are able to survive after passing through the digestive tract is a desirable feature because spores germinate and colonize faecal material producing their trapping system in which recently-hatched larvae are captured and destroyed by the fungus (Larsen *et al.*, 1995).

The present study showed for the first time the dynamics of *D. flagrans* chlamydospore passage through the gastrointestinal tract of goats. This was obtained when goats were orally inoculated once with 3.5×10^6 chlamydospores. The results of this trial were similar to those of a previous trial in sheep by Llerandi-Juárez & Mendoza de Gives (1998), who showed that chlamydospores were detected in faeces at 22 h post-inoculation and continued until 32 h post-inoculation in sheep. In goats, *D. flagrans* was detected in faeces from 21 h post-inoculation and were detectable for up to 81 h in at least two goats. Although *D. flagrans* structures were not detected in faeces at hours 3 or 9 post-inoculation, it is possible that from hour 9 to 21 the fungal structures were present in the faeces but this period was not investigated. Evidence of this was obtained in sheep by Larsen *et al.* (1998). They reported that *D. flagrans* structures can be found as soon as 8.5 h after abomasal inoculation and 12 h after oral administration. These authors used fewer chlamydospores (1.5 million) given as five smaller doses, 30 min apart (chlamydospores mixed with conidia 70%:30%) and a different vehicle of fungal administration (gelatin capsules using a stomach tube).

In the present study, goats were positive to spores or *D. flagrans* trapping structures for a longer period than the sheep used in the previous trials by Llerandi-Juárez & Mendoza de Gives (1998). Although differences in the digestive physiology between these species might have accounted for part of the differences in elimination, it is likely that the longer period of faecal spore deposition found in goats, compared to sheep, resulted from the high dose level used in this trial. With an increased dose of spores, fungi can be found for a longer period in the faeces after inoculation. The presence of trapping fungi in the cultures following inoculation in the present trial (3 to 4 days) were similar to results obtained in Malaysia (3–4 days: Chandrawathani *et al.*, 2003) but shorter than the results obtained in other trials in sheep (4 days: Larsen *et al.*, 1998; 6–7 days: Waller *et al.*, 2001; 6 days: Chandrawathani *et al.*, 2004). Chandrawathani *et al.* (2003) suggested that the transit of spores in the alimentary tract is dependent on factors likely to be related to diet and/or digesta flow of the animal. Such factors include: the amount of total feed eaten (diet + vehicle of spores), particle size, digestibility, water content of the diet and water ingestion by the animals.

The information obtained in this study contributes to the understanding of the rate of passage of spores through the

Table 1. The presence of *Duddingtonia flagrans* fungal structures in faecal cultures of goats treated with one dose of 3.5×10^6 chlamydospores per kg of body weight.

	Time (h) of sampling post-treatment											
Treated group	0	3	9	21	27	33	45	51	57	69	81	93
1	−	−	−	+	+	+	+	+	+	+	+	−
2	−	−	−	+	+	+	+	+	+	−	−	−
3	−	−	−	+	+	+	+	+	+	+	−	−
4	−	−	−	+	+	+	+	+	+	+	−	−
5	−	−	−	+	+	+	+	+	+	−	+	−
6	−	−	−	+	+	+	+	+	+	+	−	−
7	−	−	−	+	+	+	+	+	+	+	−	−

− Negative to the presence of *D. flagrans*.
+ Positive to the presence of nematodes trapped by *D. flagrans* and chlamydospores.
Note: no faecal culture in the untreated group of kids showed the presence of nematophagous fungi.

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Table 2. Mean faecal egg counts (EPG), mean larvae per gram of faeces (LPG), the percentage of larval development (% ld) and percentage of larval development reduction (% ldr) from faeces obtained from goats on days 1, 2, 3, 4 post-inoculation with *Duddingtonia flagrans* (3.5×10^6 per kg body weight) and incubated for 7 days at room temperature (31°C).

Day post inoculation	Group of goats	EPG*	LPG*	% d	% ldr
Day 1	Control	2064 ± 583a	26 ± 9a	2.0	5.3
	Treated	2250 ± 696a	24 ± 3a	1.9	
Day 2	Control	2478 ± 788a	132 ± 49a	6.6	36.0
	Treated	2828 ± 1105a	63 ± 9a	4.2	
Day 3	Control	2242 ± 952a	186 ± 75a	8.9	28.9
	Treated	1742 ± 366a	88 ± 16a	6.4	
Day 4	Control	1600 ± 632a	40 ± 13a	3.7	23.9
	Treated	1429 ± 278a	37 ± 12a	2.8	

*Different small letters in the same column within a day indicate a statistical difference ($P < 0.05$) between control and treated groups.

gastrointestinal tract of goats when fed mixed with feed. A deeper knowledge of the dynamics of transit of spores through the gastrointestinal tract with different diets might prove crucial in determining appropriate schemes of dosing and this requires information on the presence of spores in the faeces together with the number of spores eliminated in the faeces with time. The present study has shown that the transit time of spores, defined as the time from administration of 'marker' (spores) to its appearance in the faeces, can be defined in the future with a more intense monitoring scheme in the first hours post-inoculation. The other component of passage, the retention time (defined as the time in hours between appearance of 5% and 80% of marker; Van Soest, 1994) is less feasible as it needs the quantification of spores eliminated in faeces. So far, this paper has only considered the hypothesis that all spores fed to the animal pass into the faeces. Ingested material disappears from the digestive tract through two routes, digestion and passage (Van Soest, 1994). Consequently, it is important to determine that no spores are digested in order to be more accurate in the predicting the rate of passage of chlamydospores.

Experiment 2

This experiment showed that a single inoculation of 3.5×10^6 chlamydospores of *D. flagrans*, FTHO-8 strain per kg of body weight induced a reduction of infective larvae collected from faeces of infected goats. The percentage reduction of gastrointestinal nematode larvae in the treated group after a 7-day incubation period reached 36% during the second day post-inoculation. Meanwhile, the percentage reduction from faecal cultures incubated for 14 days reached around 50% on days 1 and 2 post-inoculation. However, no statistical significance was observed between inoculated and control groups in the two incubation periods (7 and 14 days). The reduction observed in the present trial was lower than that reported by Waghorn *et al.* (2003) in sheep and goats (40–93%), Paraud & Chartier (2003) in goats (84%), Paraud *et al.* (2004) in goats (86–96%) and Terrill *et al.* (2004) in goats (60.8–93.6%). The lower efficacy in the present trial may be due to different inoculation strategies used in the different trials. Waghorn *et al.* (2003) administered fungi on two consecutive days (5×10^5 per kg body weight or 2.5×10^5 per kg body weight). Paraud & Chartier (2003) used 5×10^5 per kg body weight for 7 consecutive days. Paraud *et al.* (2004) used 5×10^5 per kg for body weight 9 consecutive days. Terrill *et al.* (2004) used a maximum dose of 35×10^5 per kg body weight of *D. flagrans* but it was divided into seven daily doses of 5×10^5 per kg body weight. These dosing schemes possibly enabled a higher efficacy in their trials because fungi persist for more days in the faeces compared with the single administration used

Table 3. Mean faecal egg counts (EPG), mean larvae per gram of feaces (LPG), the percentage of larval development (% ld) and percentage of larval development reduction (% ldr) from faeces obtained from goats on days 1, 2, 3, 4 post-inoculation with *Duddingtonia flagrans* (3.5×10^6 per kg body weight) and incubated for 14 days at room temperature (31°C).

Days post inoculation	Group of goats	EPG*	LPG*	% ld	% ldr
Day 1	Control	2064 ± 583a	59 ± 16a	3.4	45.6
	Treated	2250 ± 696a	30 ± 8a	1.9	
Day 2	Control	2478 ± 788a	36 ± 13a	1.8	52.8
	Treated	2828 ± 1105a	19 ± 5a	0.9	
Day 3	Control	2242 ± 952a	35 ± 15a	1.6	0
	Treated	1742 ± 366a	62 ± 16a	3.9	
Day 4	Control	1600 ± 632a	219 ± 124a	11.1	30.6
	Treated	1429 ± 278a	107 ± 44a	7.7	

*Different small letters in the same column within a day indicate a statistical difference ($P < 0.05$) between control and treated groups.

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in this trial, even when a considerably larger dose was used. Furthermore, Terrill *et al.* (2004) also showed that fungi have more efficacy of control when offered daily compared to every second day or every third day. The information obtained under the conditions of this trial suggested that kids should be frequently fed with spores.

The percentage of larval development, which was similar to that reported in goats by Paraud & Chartier (2003) (2–26%) and Paraud *et al.* (2004) (1.55–16.2%), was lower than that reported by Terrill *et al.* (2004) in goats (3.9–100%) and Rossanigo & Gruner (1995) in sheep (36–51%). Although both experimental groups were depositing similar amounts of eggs in the faeces, there might have been factors, other than the nematophagous activity of *D. flagrans*, which might have affected hatching of the eggs or recovery of larvae from the Baermann apparatus (Terrill *et al.*, 2004). It is not possible to determine which factors were operating in this study, as all cultures were carried out under controlled laboratory conditions (temperature, humidity and oxygenation) by the same operators for the duration of the trial. However, the viability and fertility of eggs may not be easy to control and could have an affect on the number of larvae produced in the culture. The trials by Paraud & Chartier (2003) and Paraud *et al.* (2004) also suffered from a low development percentage in their cultures and these authors suggested that the strain of nematodes used in their trials could have affected their development in the faeces.

Biological control needs to match two or more life cycles. In this case, the life cycle of parasitic nematodes and nematophagous fungi are involved. Germinated fungal structures, including trapping devices, need to coincide with recently hatched larvae to establish close contact with the faecal matter. Previous trials in sheep and cattle with the FTHO-8 used incubation periods of 21 days (Mendoza de Gives *et al.*, 1998; Peña *et al.*, 2002). Such an incubation period may have allowed a longer exposure of parasitic larvae to fungal predatory activity. However, it is likely that, under field conditions in the tropics, infective larvae would leave the faecal material around day 7. Thus, a longer period of culture may result in a good percentage reduction that will not be reproduced under field conditions. In the present trial, faecal cultures incubated for 14 days showed more consistent reduction percentages, compared to cultures incubated only for 7 days. It is likely that a 14-day incubation period would also allow for a longer exposure of parasitic larvae to the fungal predatory activity.

Conclusions

Duddingtonia flagrans chlamydospores of the FTHO-8 strain survived after passing through the gastrointestinal tract of goats, preserving their trapping ability and their lethal action against infective larvae of different species of gastrointestinal parasitic nematodes. Spores were detected in goat faeces from 21 h to 81 h post-inoculation.

A single inoculation of *D. flagrans* (3.5×10^6 chlamydospores of *D. flagrans* FTHO-8 strain) per kg of body weight can induce a reduction of infective larvae collected from faeces of naturally infected goats. However, a different scheme of dosing may be needed to enhance the efficacy of *D. flagrans*.

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