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Original article

Nematophagous fungi as a biological control agent for nematode parasites of small ruminants in Malaysia: a special emphasis on *Duddingtonia flagrans*

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Abstract – Approximately 2 800 fresh dung samples from animals, mainly ruminant livestock, were screened for the presence of nematophagous fungi in Malaysia. *Arthrobotrys* spp. was noted on numerous occasions, but only one isolate of *Duddingtonia flagrans* was made. For the purposes of producing sufficient quantities of this fungus for feeding trials in sheep, various, commonly available, cheap plant materials were tested as possible growth substrates. This showed that cereal grains (wheat, millet and rice) were the best media for fungal growth. Pen feeding trials were carried out using sheep, both naturally and experimentally infected with nematode parasites (predominantely *Haemonchus contortus*), to test the efficiency of *D. flagrans* when administered either in a grain supplement, or incorporated into a feed block. These showed that the fungus survived gut passage in sheep and that dose rates of approximately 1×10^6 *D. flagrans* spores / animal / day, reduced the percentage of infective larvae developing in faecal cultures by more than 90%. These results indicate that using *D. flagrans* as a biological control agent of nematode parasites, is a promising alternative to nematode parasite control of small ruminants in Malaysia, where anthelmintic resistance is now a major problem.

nematode parasite / ruminant / tropics / biological control

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Résumé – Les champignons nématophages comme agents de contrôle des nématodes des petits ruminants en Malaisie : l'exemple de *Duddingtonia flagrans*. La présence de champignons nématophages a été recherchée dans environ 2 800 échantillons de fèces d'animaux, provenant principalement de troupeaux de ruminants, en Malaisie. *Arthrobotrys* spp. a été trouvé à de nombreuses occasions, mais seul un isolat de *Duddingtonia flagrans* a été détecté. Afin de produire des quantités suffisantes de ce champignon pour des tests dans l'alimentation du mouton, plusieurs plantes facilement disponibles et bon marché ont été testées en tant que substrat de croissance. Ces essais ont montré que les grains de céréales (blé, millet et riz) constituaient le meilleur milieu pour la croissance des champignons. Des essais alimentaires en enclos ont été menés en utilisant des moutons infestés naturellement ou expérimentalement par les nématodes parasites (principalement *Haemonchus contortus*), afin de tester l'efficacité de *D. flagrans* administré soit dans un supplément de grains, soit incorporé dans un bloc de nourriture. Les résultats ont montré que le champignon survivait au passage dans les intestins de mouton et que des doses d'environ 10^6 spores de *D. flagrans* par animal et par jour réduisaient de plus de 90 % le pourcentage de larves infectieuses se développant dans les cultures fécales. Ces résultats montrent que le principe de l'utilisation *D. flagrans* comme agent biologique de contrôle des nématodes parasites des petits ruminants est une alternative prometteuse en Malaisie, où la résistance aux anthelmintiques est aujourd'hui un problème majeur.

parasite nématode / ruminant / tropique / contrôle biologique

1. INTRODUCTION

Helminth parasite and respiratory infections are the main causes of disease and mortality in small ruminant (sheep and goat) production systems in Malaysia [17]. These problems have become more serious with the depletion of land and grazing resources within the country and the inevitable intensification of livestock farming systems. Farmers have relied heavily on the intensive use of commercial anthelmintic preparations to control nematode parasites in their flocks. As a consequence, very high levels of anthelmintic resistance have now been reported, particularly in large commercial enterprises in Malaysia [3–6, 15, 18]. In an attempt to develop methods of parasite control, which are alternative to the intensive use of anthelmintics, pasture management systems and the use of low-dose anthelmintic medicated feed blocks have been tested [2]. Although successful, these have had minimal appeal and thus acceptance by farmers, who are more attuned to suppressive (every 4–6 weeks) use of anthelmintic drugs. However, because of the seriousness of the anthelmintic resistance problem, attitudes are now beginning to change. Coupled with this is the new awareness of environmental safety amongst producers and consumers alike in Malaysia and now the government is promoting agricultural practices which do not rely on intensive chemical control [1].

One exciting recent development in the non-chemical approach to parasite control is the exploitation of the nematode-destroying capacities of certain fungi, especially *Duddingtonia flagrans* (for reviews see: [8, 11, 21, 22]). This study was aimed initially at a survey in Malaysia to detect and isolate a local strain of *D. flagrans* and then to assess its potential as a biological control agent of nematode parasites of small ruminants under local conditions.

2. MATERIALS AND METHODS

2.1. Survey, isolation and identification of nematophagous fungi

During January 1997 to November 2000, a total of 2 805 faecal samples derived from animals of the entire peninsula of Malaysia were submitted to the Department of Parasitology at the Veterinary Research Institute,

Ipoh, Malaysia, for routine parasitological examination (estimation of helminth and coccidia infections). The main source was from the Central Zone (Perak, Selangor, Pahang – 2 487 samples), but material was also obtained from the Northern Zone (Perlis, Kedah, Kelantan, Terengganu – 160 samples) and the Southern Zone (Johore, Malacca, N. Sembilan – 158 samples). These were collected from cattle, goats, sheep, buffaloes, horses, zoo animals, dogs, deer and pigs. Surplus material was then used for screening for the presence of nematophagous fungi in the fresh dung. The procedures undertaken were generally in accordance with those previously reported by workers in Australia [12]. Basically, 2–5 g faeces were mashed and cultured on water agar (2%) petri dishes and incubated at room temperature (30–35 °C). Infective larvae (L3) of *Haemonchus contortus* (approx. 200) in a concentrated drop of water, were added to each of the plates to serve as bait for any nematophagus fungi that may have been present. The plates were examined daily, or on alternate days, to determine the presence of trapped larvae. The examinations were first made after three days of incubation. Spores of the nematode trapping fungi were then transferred carefully onto separate petri dishes containing sheep faecal agar, as previously described [20]. Sub-cultures were then carried out until pure cultures were obtained, which were thereafter maintained on cornmeal agar (2%) and were stored at 4 °C. Identification of nematophagous fungi was based on the trapping mechanism involved and the morphology of conidiophores, conidia and the presence of chlamydospores [16, 19].

2.2. *Duddingtonia flagrans* - culturing and chlamydospore production

With the successful isolation of *D. flagrans*, efforts were made to culture this fungus on a variety of commonly available, cheap, locally produced plant materials to obtain large quantities of fungal material for in vivo studies in sheep. Ten plant materials were used to determine the best possible substrate(s) for this purpose. In a preliminary trial, 200 g of the media in four replicates were weighed, air dried and placed in 1 L flasks together with 200 mL of distilled water. The flasks were sealed with a cotton wool plug, autoclaved at 15lb pressure at 121 °C for 10–15 min, and were then cooled overnight. Pure cultures of *D. flagrans* grown for 6 weeks on cornmeal agar in petri dishes (9 cm diam.) were then cut into small cubes of 2–3 cm and were inoculated into these flasks under sterile conditions. One plate of agar culture was the amount of fungal material inoculated into each flask, with 4 flasks for each media. The flasks were kept at room temperature (25–35 °C) and were shaken daily in an attempt to break-up and spread the fungal growth within the media. After 2 weeks, the media were subjectively assessed in order to determine the extent of fungal growth.

Further culturing procedures were conducted in an incubator (20 °C) to determine the optimal moisture levels in rice, wheat and millet as the culture media. For each cereal grain, four replicate cultures were prepared at each of the following water: grain ratios – 40:60, 50:50 and 60:40. The cultures were incubated for six weeks and then removed from the flasks, the fungal/grain clumps were separated by hand into a single layer of grains on plastic sheets in an isolated room. This was then air-dried using an electric fan, at ambient temperatures. After 3–5 days of drying, an estimate of the chlamydospore production was made. A set weight (10 g) of grain material was lightly crushed with a mortar and pestle in distilled water, sieved with a tea sieve, to separate the bigger particles, and the strained material collected for estimation of chlamydospore concentration with the use of a haemocytometer. Estimates were repeated 5 times to assure a reasonable degree of accuracy.

2.3. In-vivo feeding trials

Feeding trials of fungal material were conducted using sheep (Border Leicester/Merino crossbreds) approximately 6–8 months of age, weighing approximately 25 kg. In the first trial, the sheep had natural (paddock acquired) parasite infections, consisting mainly of *Haemonchus contortus*. These animals were kept in individual pens and were fed daily for 5 days with *D. flagrans* spores that were produced on wheat grain (processed at 20 °C for 6 weeks with a 50:50 water:grain ratio), at 3 different spore dose rates (1.25×10^6, 2.5×10^6, 6.25×10^6), with 2 animals at each dose rate. The fungal dose was mixed with a small amount of concentrate and was fed to the animals in a feed trough every morning, prior to them having access to their normal rations. Throughout these studies, sheep were fed with grass *ad libitum,* and 500 g of commercial concentrate daily. Daily nematode faecal nematode egg counts and larval cultures were conducted during the fungal feeding period, as well as pre- and post-feeding. In addition, cultures were made in order to estimate the presence of fungus. This was achieved by placing approximately 3 g of mashed faeces onto a water agar plate in the form of a cross, to which a drop of water containing *H. contortus* L3 (approx. 200) was added. The plate was examined daily for 3–6 days for the presence of characteristic features of *D. flagrans* [16, 19]. Feeding of fungus was conducted for 5 days and the faecal procedures continued for an additional week.

In the second in vivo study, four sheep approximately 8–12 months of age and weighing 25–28 kg were chosen. In contrast to the first study, where the sheep had naturally acquired infections, all four sheep were experimentally infected with 500 *H. contortus* L3 per animal, on alternate days, for three occasions (1 500 L3/animal). Four weeks after larval dosing, all sheep had positive faecal egg counts and this was the commencement time for the trial. Two animals were allocated to the fungal group and the other two to the control group that were not fed with fungus material. The sheep were kept in individual pens. The fresh fungal material was divided into 60 g (approx. 1×10^6 spores) amounts, which was fed individually to the fungal group in feed troughs for 5 consecutive days. When the sheep had consumed the entire fungal supplement, they were provided with grass pellets *ad libitum*. No fresh grass was given throughout the experiment.

2.4. Fungal feed block trial

A group of 6 female sheep, 6–8 months of age, were identified based on their high nematode faecal egg counts (> 1 000 epg). They were provided each week with a standard 4 kg urea-molasses small ruminant block consisting of a range of ingredients [10], but with only 5% urea. These blocks were offered to the sheep, 4 weeks prior to the start of this trial to adapt them to a voluntary consumption of this supplement. Prototype fungal feed blocks were prepared by incorporating wheat media supporting the growth of *D. flagrans* for 6 weeks into standard formula urea-molasses blocks, at a rate of 100 g fungal material per block of 4 kg. The fungal material contained 1×10^5 spores/g of dried grains. The blocks were made one week before the start of the trial at the Nutrition Unit of the Malaysian Agricultural Research and Development Institute, consigned to the Veterinary Research Institute, where they were stored in a cold room (4 °C) throughout the course of the trial. These fungal blocks replaced the standard blocks and were offered to the group of sheep for a total of 7 weeks. A new block was placed in the group pen at the start of each week. Throughout the trial, intake of block, individual faecal egg counts and larval cultures were recorded on a daily basis. Duplicate faecal sample cultures were made to detect the presence of *D. flagrans* on 3 occasions (Monday, Wednesday and Friday)

each week. In addition, approximately 1 g of material was taken from the new blocks at the start of each week, prior to it being placed in the sheep pen. This sample was crushed and placed on a water agar plate, was kept at room temperature (25 °C) and was observed daily for fungal growth for 14 days.

3. RESULTS

3.1. Survey, isolation and characterization of nematophagous fungi

The distribution of faecal samples according to the animal species is shown in Table I, where it can be seen that the vast majority of samples were derived from cattle, sheep and goats.

A large proportion of the nematophagous fungi that was detected fulfilled the classification criteria of *Arthrobotrys* spp. These were based on observations of the morphology of the conidia and the specialized trapping organs. Other nematode trapping fungal species were noted, but were not isolated for specific identification, except for one isolate that possessed all the taxonomic characteristics of *D. flagrans.* This isolate originated from sheep faeces from a privately owned farm, located at Gopeng (the Central Zone), which also maintained goats, dairy cattle and buffaloes in a mixed farming enterprise. Detailed taxonomic observations and measurements were made of this species. When grown on water agar, the colony was found to be sparsely spreading and whitish in color. The hyphae were hyaline, septate and branching and the conidiophores were approximately 150 μm long. Conidia were obovoidal/ellipsoidal 25–50 × 10–15 μm. In addition, abundant spherical chlamydospores were present in colonies when aged approximately 3 weeks

Table I. Number of faecal samples from various species of animals in Malaysia that were screened for the presence of nematophagous fungi over a period of 4 years (1997–2000) and the percentage that were found to be positive.

Host species	Year					Nematophagous fungi
	1997	1998	1999	2000	Total	No (%)[a]
Buffalo	13	6	52	17	88	20 (23)
Cattle	159	158	129	155	601	91 (15)
Deer	2	0	0	5	7	2 (29)
Dog	3	0	7	1	11	0 (0)
Goat	170	255	120	268	813	285 (35)
Horse	7	27	105	22	161	13 (8)
Pig	13	0	15	15	43	0 (0)
Sheep	83	167	350	410	1 010	455 (45)
Zoo animals[b]	17	22	32	0	71	3 (4)
Total	467	635	810	893	2 805	779 (28)

[a] Number (%) of samples which were positive for nematophagous fungi.
[b] Primates, elephant, rhino, tapir.

(Fig. 1). By 6 weeks, the cultures began to degenerate with little hyphal growth but many chlamydospores were present.

3.2. *D. flagrans* chlamydospore production using various local media

The types of media selected for testing of a potential solid state media for the growth of *D. flagrans,* are shown in Table II.

In the seven types of media, either the cultures tended to set into a solid, pudding-like mass, so that they could not be properly mixed (green beans, small red beans, coconut meal), or the growth of the fungi was very poor (large red beans, soya beans, black beans), or undetectable (oil palm kernels). In 3 types of media, namely chicken feed (second quality processed wheat grain), bird seed (processed millet grain), and unprocessed whole grain padi (rice), the fungal growth was regarded as being good, or excellent. This was evidenced by the profuse production of white mycelia particularly early (2–4 weeks) in the culturing process. Importantly from the standpoint of distributing the fungi throughout the culture mass in the flasks, daily shaking could break up the cultures. The cereal grain cultures with different proportions of grain:water incubated for 6 weeks, showed considerable variation in the production of *D. flagrans* chlamydospores between replicates for each cereal and for each water content (Tab. III). The optimal grain:water concentrations for chlamydospore production was 60:40 for wheat

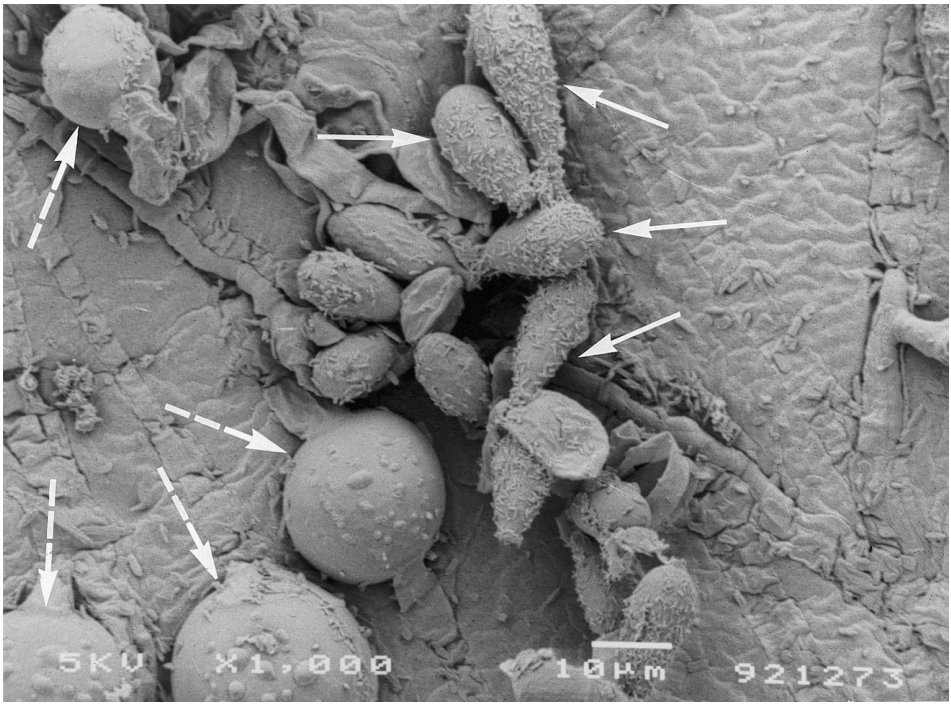

Figure 1. Scanning electron micrograph of conidia and chlamydospores of a 6-week culture of the Malaysian isolate of *Duddingtonia flagrans*, growing on 2% cornmeal agar. (Broken arrow denotes chlamydospores; solid arrow denotes conidia).

Table II. Culture media for *Duddingtonia flagrans.*

Types of Media Tested[a]	Growth characteristics after 2 weeks
Green peas (*Phaseolus aureus)*	Not suitable - media mushy / solid lumps
Red beans (large) (*P. calcaratus)*	Not suitable - particles too large
Red beans (small) (*P. calcaratus*)	Not suitable - media mushy / solid lumps
Soya beans (*Glysine soya*)	Not suitable - fungal growth limited
Black beans (*Cajanus cajan*)	Not suitable - fungal growth limited
Oil palm kernels (*Elaeis guineensis*)	Not suitable - no growth
Coconut meal (*Cocos mucifera*)	Not suitable - media mushy / solid
Millet grain *(Sorghum vulgare)*	Suitable - media supports good growth
Padi rice *(Oryza sativa)*	Suitable - media supports good growth
Wheat grain (*Triticum arestivum*)	Suitable - media supports good growth

[a]50% media:50% water, cultured for 2 weeks at 23–25 ^{o}C (4 replicates).

Table III. Cereal Grain Cultures for *Duddingtonia flagrans* chlamydospore production at different proportions of grain:water, cultured for 6 weeks at 20 ^{o}C.

	Grain:water		
Grain	60:40	50:50	40:60
Padi rice	66 500 ± 63 100[a]	50 000 ± 33 600	30 875 ± 23 100
Millet grain	63 750 ± 24 950	180 000 ± 65 000	59 000 ± 12 750
Wheat grain	207 500 ± 64 000	100 000 ± 22 200	141 250 ± 67 400

[a]Chlamydospores/g DM (±SD) – 4 replicates.

and padi rice, but 50:50 for millet grain. Millet and wheat grain tended to be the best substrate and wheat grain was selected for future work because it was cheap and readily available.

3.3. In vivo feeding trials

3.3.1. Trial 1 (Sheep naturally infected with H. contortus*)*

Feeding fungi at all three dose rates showed a dramatic reduction in the percentage of infective larvae that succeed in developing from nematode eggs in the faeces of all sheep incubated under ideal culturing conditions (Fig. 2). There was evidence of a dose-response, with the degree of reduction in larval development increasing with fungal spore rate. In addition, *D. flagrans* was detected in the faeces from all lambs in the three groups, 24 hours after fungal feeding began (Fig. 2). In group A and B, the fungus was continually isolated till day 7 and day 8, which was 1 and 2 days post feeding respectively, whereas in group C it was isolated until day 10 (4 days post feeding).

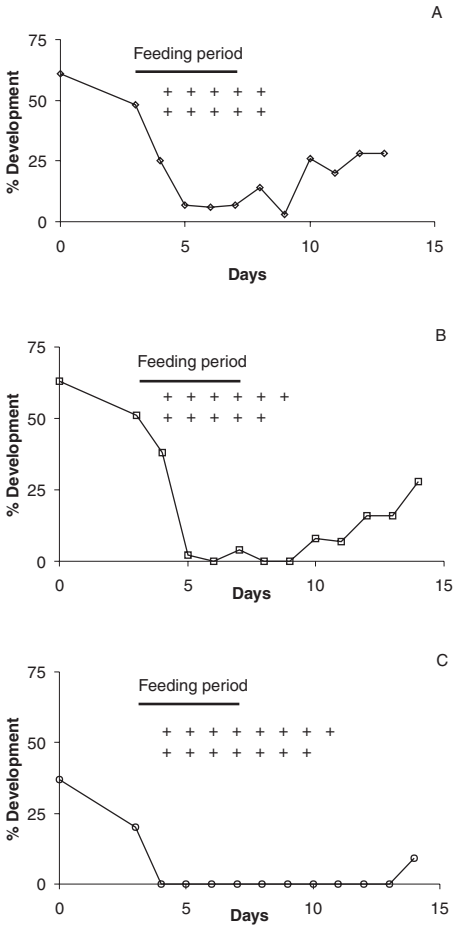

Figure 2. Mean percentage development of nematode eggs to infective larvae in the faeces of lambs fed: 1.25×10^6 (Group **A**), 2.5×10^6 (Group **B**) or 6.25×10^6 (Group **C**) *D. flagrans* chlamydospores/animal/day.
Number of + represents the presence of *D. flagrans* in faecal cultures for each lamb at each dose rate.

3.3.2. Trial 2 (Sheep experimentally infected with H. contortus*)*

Recovery of infective larvae was maintained at approximately 50–70% of the nematode faecal egg counts in the control group, receiving no fungal material (Fig. 3). Twenty-four hours after fungal feeding commenced, the fungal fed group showed a marked reduction (97%–100%) in larval recovery, which continued for two days post-feeding. *D. flagrans* was continuously isolated from the dung of the sheep fed the fungal supplement during the period of larval reduction in the cultures (Fig. 3).

3.4. Fungal block trial

It was found that during the block-feeding acclimatization period, the group of 6 sheep consumed unmedicated blocks in approximately 2 days. However, it was found that the group completely consumed the fungal blocks on the first day of the week throughout the 7 weeks of the trial. There was no indication that this rapid block consumption had any detrimental effects on the sheep, such as urea toxicity, since all the animals remained healthy and their appetite was not depressed during the course of the study. Thus, the consumption of the block was on average 660 g/sheep/week, equivalent to 1.6×10^6 spores per week.

Faecal egg counts of the sheep showed considerable fluctuations during the 7 week trial period. Differentiation of the larval cultures showed that *H. contortus* was the

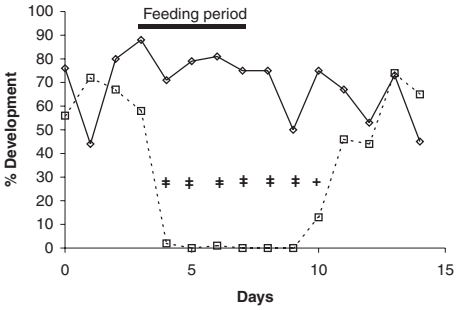

Figure 3. Mean percentage development of *Haemonchus contortus* eggs to infective larvae in the faeces of lambs, either untreated, or fed 1×10^6 chlamydospores *D. flagrans*/animal/day.
Number of + represents the presence of *D. flagrans* in faecal cultures for each lamb in the fungal treatment group.

main species present (≅ 90%), with *Trichostrongylus*, *Oesophagostomum* and *Bunostomum* spp. comprising the remaining minor species. During week 1 of the trial, infective larvae were recovered for the 2 days of sampling with the highest number of L3 on the first day (1 500 L3/g faeces) (Fig. 4). The numbers decreased drastically on the second sampling day (200 L3/g faeces). After this, there was a total failure of cultures to yield L3 during weeks 2, 3 and 4. Although there was an increase in L3 recovery in weeks 5, 6 and 7, this did not reach the same level as at the beginning of the trial. Growth of *D. flagrans* on faecal samples was observed erratically throughout the trial. Difficulties were experienced with cultures made up from samples of the fungal blocks. Positive identification of *D. flagrans* was only made on three occasions. This was mainly due to the precocious development of the unidentified fungi, causing profuse overgrowth of the plates within 7–10 days of incubation, thus obscuring and inhibiting the growth of *D. flagrans*.

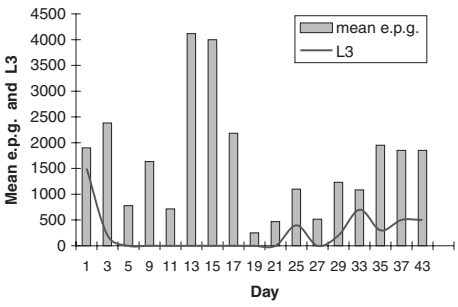

Figure 4. Mean faecal egg counts and percentage larval development in 6 lambs offered a supplementary feeding block containing *D. flagrans* chlamydospores for a 7-week trial period.

4. DISCUSSION

Considerable efforts were required to make the first isolate of *D. flagrans* in Malaysia. Although a relatively large percentage of the approximately 2 800 fresh dung samples of ruminants examined contained nematophagous fungi, just one isolate of *D. flagrans* was identified and successfully isolated. The only other large-scale surveys for the presence of nematophagous fungi in fresh faeces of livestock were those conducted in Australia [12] and Fiji [14]. In the former study, 17 isolates of *D. flagrans* were made from 1742 dung samples mainly from ruminants [12], whereas in the latter, 2500 sheep and goat samples failed to yield an isolate of this fungus [14]. The greater success rate in the Australian survey than in either the Fijian work or in this study, suggests that *D. flagrans* is relatively less abundant in tropical livestock producing regions, or less able to compete in faecal cultures where *Arthrobotrys* spp. is more prevalent. As in the other two surveys, we concentrated on samples of fresh dung because in order to exploit such fungi as bio-control agents against the free-living stages of nematode parasites of livestock, the fungus needs to survive gut passage of the livestock. This allows for the opportunity of incorporating the fungal material into the feed of animals. Although a large percentage of samples yielded *Arthrobotrys* spp., previous comparative studies have shown that much greater quantities of *Arthrobotrys* spp. conidia need to be fed to animals to obtain a similar in vivo efficacy against the free-living stages of parasites as with *D. flagrans* chlamydospores [7].

As in the studies in Denmark [8, 9] and Australia [13, 23], the Malaysian *D. flagrans* isolate was found to grow most satisfactorily on moist, sterilized cereal grains. Autoclaving the moistened green peas and small red beans caused them to adhere into sticky solid masses, and the hard pericarp of large red beans, soya beans and black beans prevented the complete absorption of the added water. Possibly better results could have been achieved with these media if less water was added (green peas

and small red beans), or they were slightly crushed (other beans). The lack of growth on coconut meal and oil palm kernels is attributed to the high proportion of plant oils in these substrates. The purpose of investigating a range of possible growth substrates for *D. flagrans* was to determine the best of the locally available, cheap plant by-products for scaling-up the production of *D. flagrans* to provide sufficient material to conduct large-scale field studies on biological control of nematode parasites of ruminants in Malaysia.

Although the Malaysian isolate of *D. flagrans* grew profusely, as evidenced by the abundant white mycelial growth on the grain substrates, its production of chlamydospores was much lower after 6 weeks of incubation ($\cong 1 \times 10^5$/g dried grain), compared with Australian isolates ($\cong 5 \times 10^5$/g dried grain, after 4–5 weeks incubation [23]) and Danish isolates ($\cong 2.5 \times 10^5$ / grain of barley incubated for 2 weeks at 20–22 ^{o}C [9]). Intra-species variation of nematophagous fungi in various morphological and physiological characteristics, including the production of conidia and/or chlamydospores, is well known [16, 19].

The feeding trials demonstrated that the Malaysian isolate of *D. flagrans* was highly efficient in reducing the number of *H. contortus* L3 that succeed in developing in faecal cultures of infected animals. In the first trial, pairs of sheep were administered either 1.5×10^6, 2.5×10^6, or 6.25×10^6 chlamydospores per animal per day for 5 days. All treatments showed a profound, dose-dependent reduction in larval development, which became evident 24 hours after the commencement of fungal dosing.

Concomitant with this reduction was the presence of *D. flagrans* in faecal cultures. In the second trial, larval development in the faeces of the control lambs remained relatively constant (approx. 50–70% of egg count), whereas in the fungal group, larval numbers were almost eliminated throughout the 5-day fungal feeding period. Fungal presence in dung persisted for 4 days after the cessation of feeding and with its disappearance, the number of larvae in the cultures regained the pre-treatment levels. These results agreed with studies where sustained infusion via the cannulae of *D. flagrans* chlamydospores into the rumen of lambs infected with *Trichostrongylus colubriformis*, showed that between 5×10^5 and 1×10^6 chlamydospores/animal/day result in a substantial (> 80%) reduction in the number of infective larvae in faecal cultures [13].

To extend the means by which *D. flagrans* could be used as a practical means of fungal delivery to animals, a prototype formulation of supplementary feed blocks containing fungal material were prepared and tested. In this trial, a group of nematode infected young sheep were offered a urea-molasses block containing fungal material, designed to deliver 1.6×10^6 spores/animal/week. Prior block consumption data indicated that it should have taken the animals approximately 2 days to consume the 4 kg blocks offered. However, the formulation obviously made the blocks highly attractive to the animals since they were consumed within the first day of offer. Infective larvae were eliminated from faecal cultures within 4 days of the start of block feeding and for the following 3 weeks. Although gut passage of the chlamydospores would have extended over several days, as demonstrated in the previous in vivo studies, it is difficult to explain the even more prolonged effects on the suppression of infective larval yields from faecal cultures, which could be attributed to fungi delivered by these means, except that ingestion of urea is known to reduce rumen motility and thus digesta flow through the gut. Infective larvae started to increase in the latter 3 weeks of this block feeding trial. Even though we were unable to estimate the viability of *D. flagrans* spores in the stored blocks because of overgrowth of other fungal contaminates, this suggests that the viability

of these spores deteriorated with increasing block storage. This finding was consistent with previous studies of prototype fungal feed blocks, where spore viability declined with the storage in block formulations where water was a significant component [23]. This is attributed to the likely initiation of germination processes by the chlamydospores, which would render them susceptible to the harsh environmental conditions of the ruminant gut.

Taken together, these results are strongly supportive of the concept of developing a means by which *D. flagrans* could be used as a biological control agent of nematode parasites of small ruminants in Malaysia. Although considerable work needs to be done to produce a practical means of fungal deployment that will hopefully appeal to farmers, this work is very timely and important. This is because it offers the possibility of a non-chemotherapeutic approach to the control of parasites, which are now developing high levels of resistance to anthelmintic drugs in sheep and goat farms in Malaysia.

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