

SHORT COMMUNICATION

A PRELIMINARY STUDY TO DETERMINE THE EFFICACY OF A NEMATOPHAGOUS FUNGUS, *ARTHROBOTRYS OLIGOSPORA*, AGAINST NEMATODE LARVAE IN CATTLE AND GOAT DUNG

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Currently, gastrointestinal nematodes are considered to be a major cause of health concern in cattle¹ and goats² in Sri Lanka. Control of gastrointestinal nematodes, which is of major importance in ensuring the sustainability of ruminant production, is currently achieved by the use of anthelmintics. Frequent use of anthelmintics, however, has led to the development of resistance in gastrointestinal nematodes of goats³ in the dry zone where goat farming is a popular livestock enterprise among rural communities. The increasing incidence of anthelmintic resistance necessitates the development of alternative strategies for parasite control. The nematophagous fungi, which have developed a unique ability to prey on live nematodes,⁴ present an opportunity to control the developing stages of gastrointestinal nematodes, thus preventing the rise of infective third stage larvae on pasture.⁵ A bioassay was conducted in order to study the *in vitro* efficacy of native, *Arthrobotrys oligospora*, a nematophagous fungus, originally isolated from cattle dung,⁶ in reducing the infective third stage larvae (L₃) of cattle and goat gastrointestinal nematodes.

To obtain the fungal spores in large quantities for the study, 7 day old fungus on 0.6% Corn meal agar (CMA) was cultivated on barley grains for 3 weeks.⁷ The fungal elements were harvested by adding distilled water and gently scraping the surface of the grains into a petri dish. The enumeration of spores was carried out systematically moving a petri dish (9 cm diameter and scored with lines to facilitate counting) back and forth until the entire area has been examined under a dissecting microscope, thus excluding the possibility of underestimation of spore concentration. The number of spores in 1ml of fluid was adjusted to 250,000. However, the method of collection of conidia resulted in addition of viable germinating mycelial fragments also in the suspension and the number of potential germinating units in the suspension could therefore have been under estimated.

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The faecal samples collected from cattle and goats, with natural gastrointestinal nematode infections (*Haemonchus* spp.; *Trichostrongylus* spp.; *Bunostomum* spp. and *Oesophagostomum* spp.) were pooled separately and mixed thoroughly by breaking up the faeces finely using a wooden spatula. The mean nematode egg counts (egg), as determined by Modified McMaster technique⁸ in five different samples taken separately, were 520±26 and 435±46 for cattle and goats, respectively.

Two experiments were conducted, one for cattle and one for goat gastrointestinal nematodes, using a spore concentration of 500,000 per 5g faecal sample. The ratio of spores per nematode eggs attained thus were 192 and 230 for cattle and goat faeces, respectively. In order to determine an appropriate spore concentration, 10, 100 and 500 spores per egg concentrations in faeces obtained from a goat with pure *Haemonchus contortus* infection were tested previously and 65%, 80%, and 97% reduction of infective *H. contortus* larvae respectively were observed, suggesting that the concentration of conidia used in the present study was unusually high.

Each of the 20 cattle and 20 goat faecal samples (5 g each) that derived from the pooled faeces with known amounts of nematode eggs were placed in individual plastic containers with perforated lids, and were treated with 2 ml of *A. oligospora* suspension (250,000 spores/ml) while each of the 20 cattle and 20 goat faecal samples (5 g each) that acted as controls were treated with 2 ml of distilled water. When cultures were too wet, dried sterile bovine faeces powder was added until the correct consistency was obtained.⁸ The samples were individually homogenized using a wooden spatula and placed in an incubator at 27 °C for 10 days. Faecal cultures were stirred daily and any shortage of moisture was noted, the required consistency was achieved by adding water.⁸ The L₃ larvae were harvested by the Baermann technique⁸, stained with 45% iodine, identified and counted.

The L₃ counts in *A. oligospora* treated cultures were compared with that of the controls using Student's 't' test by a computer statistical package, MINITAB (Release 10.1, Minitab Inc.). Before analyses L₃ counts were log transformed in order to stabilize variance. The fact that means of L₃ counts were substantially lower than the respective variance (SD²) suggested the need of data transformation. The fact that the means were higher in Log transformed data than the variance indicated that the transformation was effective. Comparisons were considered significant at p<0.05. Efficacy of *A. oligospora* in reducing L₃ numbers was assessed by calculating the percentage reduction for cattle and goat gastrointestinal nematodes separately and approximate 95% confidence intervals (CI) were calculated for the percentage reduction.⁹

Faecal cultures of control cattle showed L₃ larvae of *Haemonchus* (68%), *Trichostrongylus* (17%), *Bunostomum* (11%) and *Oesophagostomum* (4%) whilst that

of control goat had *Haemonchus* (61%), *Trichostrongylus* (37%) and *Oesophagostomum* (3%).

The mean number of L₃ recovered from treated and control faecal cultures of cattle were 30±20 and 319±115 respectively whilst that of goats were 18±7 and 227±19 respectively. The addition of *A. oligospora*, significantly reduced the recovery of gastrointestinal nematode L₃ in faeces derived from both cattle 90.5% (CI=86.4-93.3) and goat 92% (CI=90-93.7) at p<0.0001. It is necessary to test the passage of this fungal isolate through the gut and subsequent activity of the fungus in the dung pat in order to be able to assess if it has the potential to become of practical use as a biological control agent of gastrointestinal nematodes of ruminants.

Unexpectedly higher concentrations of spores per nematode egg were used in this study. In an experimental design similar to the present one, it has been demonstrated that an almost equal reduction of cyathostome L₃ in horse faeces occurred but at a 10-100 *A. oligospora* spores per egg ratio.⁹ A 98% reduction of the larval population of *H. contortus* has been observed in sheep faecal cultures¹⁰ at the rate of 20,000 conidia per gram of faeces where egg was 11680 at *A. oligospora* addition. In a different context, as low as 250 to 500 conidia of *A. oligospora* per gram of faeces where egg was not indicated, have reduced *H. contortus* larvae significantly in sheep faecal culture.¹¹ In the present study, a ratio of 193 and 230 spores per egg was selected for cattle and goat gastrointestinal nematode respectively, based on a preliminary observation made with pure *H. contortus* infection and the requirement of unexpectedly high concentration of spores was unexplainable. However, it may reflect a possibility that as has been indicated by studies carried out elsewhere,¹¹⁻¹² the activity of *A. oligospora* would have been low against *H. contortus*. In the preliminary study carried out to establish the spore concentration, pure *H. contortus* infection was used and it is the dominant gastrointestinal nematode tested in the present study.

Ideally, species of nematophagous fungi whose spores can withstand the stress of digestion may be used as a feed additive for livestock.⁵ In this way, viable spores will be present by the gastrointestinal nematode eggs, trapping the larvae as they develop at the dung pat micro environment thus reducing the pasture contamination. The ability of *A. oligospora* isolates to maintain the viability after passage through the gastrointestinal tract has been reported as poor when compared to *Dadlingtonia flagrans*.^{4,7,13} However, the strain assessed in this study was originally isolated from directly obtained cattle faecal samples indicating that the strain is capable of going through the gastrointestinal tract of cattle without losing its viability.¹⁴ This observation could be supported by the previous observations made where passage of *A. oligospora* spores through the gastrointestinal tracts of ruminants has been studied.¹⁵⁻¹⁷ However, the gut survival characteristic of *A. oligospora* appears to be dose dependent and inconsistent.^{14,17} In addition to the capability of gut survival of the fungus, presence of an effective concentration of the conidia of *A. oligospora* in

the dung pat is also important for the beginning of its activity against gastrointestinal nematodes.¹⁸ The inconsistent passage of the unprotected *A. oligospora* through the gastrointestinal tract of ruminants, and the necessity of an effective concentration of fungal spores in the dung pat have reduced its interest as a possible feed additive to be used as a biological control agent.

There is a possibility, however, that the unprotected conidia of *A. oligospora* can be applied directly in the pens as a spray or dry powder for the control of *Strongyloides papillosus* preventing massive percutaneous infections in young livestock as has been suggested by other studies carried out in India¹⁹ and Malaysia.²⁰ Infection by *S. papillosus* that is capable of causing economic losses,²¹ appears to be the most prevalent gastrointestinal nematode in calves raised intensively in the intermediate zone of Sri Lanka.²² As has been observed in temperate conditions, the activity of *A. oligospora* against gastrointestinal nematodes in the field is considerable and reduced the infection in grazing calves by about 37% when compared to those raised in control pens.²³ However, this approach needs to be tested under local conditions in order to make use of *A. oligospora* as a biological control agent against *S. papillosus*.

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