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Milani Araujo, Juliana; Victor de Araújo, Jackson; Ribeiro Braga, Fabio; de Oliveira
Tavela, Alexandre; Ferreira, Sebastião Rodrigo; de Freitas Soares, Filipe Elias; Ribeiro
Carvalho, Giovanni

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Control of *Strongyloides westeri* by nematophagous fungi after passage through the gastrointestinal tract of donkeys

Controle de *Strongyloides westeri* por fungos nematófagos após trânsito gastrintestinal em jumentas

Juliana Milani Araujo^{1*}; Jackson Victor de Araújo¹; Fabio Ribeiro Braga¹; Alexandre de Oliveira Tavela¹; Sebastião Rodrigo Ferreira¹; Filipe Elias de Freitas Soares²; Giovanni Ribeiro Carvalho³

¹Department of Veterinary Medicine, Federal University of Viçosa – UFV, Viçosa, MG, Brasil

²Department of Biochemistry and Molecular Biology, Federal University of Viçosa – UFV, Viçosa, MG, Brasil

³Department of Zootechnics, Federal University of Viçosa – UFV, Viçosa, MG, Brasil

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Abstract

Strongyloides westeri is the most prevalent nematode among equines aged up to four months and causes gastrointestinal disorders. The objective of this study was to observe the control of infective *S. westeri* larvae (L₃) by the nematophagous fungi *Duddingtonia flagrans* (AC001) and *Monacrosporium thaumasium* (NF34) after passage through the gastrointestinal tract of female donkeys. Twelve dewormed female donkeys that were kept in stables were used. Two treatment groups each comprising four animals received orally 100 g of pellets made of sodium alginate matrix containing a mycelial mass of either *D. flagrans* (AC001) or *M. thaumasium* (NF34). The control group consisted of four animals that received pellets without fungus. Feces samples were then collected from the animal groups at different times (after 12, 24, 48 and 72 hours). These feces were placed in Petri dishes containing 2% water-agar medium and 1000 L₃ of *S. westeri*. AC001 and NF34 isolates showed the ability to destroy the L₃, after gastrointestinal transit, thus demonstrating their viability and predatory activity.

Keywords: Nematophagous fungi, *Duddingtonia flagrans*, *Monacrosporium thaumasium*, *Strongyloides westeri*, female donkeys.

Resumo

O *Strongyloides westeri* é o nematóide de maior prevalência entre equídeos com idade até quatro meses, causando distúrbios gastrintestinais. O objetivo do presente trabalho foi observar o controle de larvas infectantes (L₃) de *Strongyloides westeri* pelos fungos nematófagos *Duddingtonia flagrans* (AC001) e *Monacrosporium thaumasium* (NF34) após trânsito gastrintestinal em jumentas. Foram utilizadas 12 jumentas, estabuladas e previamente vermifugadas. A seguir, dois grupos tratados, contendo cada um 4 animais receberam por via oral 100 g de péletes em matriz de alginato de sódio, contendo massa miceliana dos fungos *D. flagrans* (AC001) ou *M. thaumasium* (NF34). O grupo controle foi constituído de 4 animais que receberam péletes sem fungo. A seguir, amostras de fezes dos grupos de animais foram coletadas em distintos intervalos de horas (12, 24, 48 e 72). Essas fezes foram vertidas em placas de Petri contendo meio sólido ágar-água 2% e 1000 L₃ de *S. westeri*. Os isolados AC001 e NF34 apresentaram capacidade de destruir as L₃ após o trânsito, demonstrando sua viabilidade e atividade predatória.

Palavras-chave: Fungos nematófagos, *Duddingtonia flagrans*, *Monacrosporium thaumasium*, *Strongyloides westeri*, jumentas.

According to FAO (2008 apud MORROW et al., 2011), there are approximately 43 million donkeys (*Equus asinus*) in the world, serving mainly as a transportation resource in developing countries (PRITCHARD et al., 2005). Getachew et al. (2010) reported

that many helminth species are found in these animals and cause direct damage to their health. Nevertheless, there are insufficient studies with data on strategic control of gastrointestinal helminthic parasites that affect this equine species (MATTHEE et al., 2002; VENEZIANO et al., 2011). Within this context, *Strongyloides westeri* is a relatively frequent nematode in young animals that may cause gastrointestinal tract disorders (URQUHART et al., 1998). Although equines generally develop satisfactory immunity to this

*Corresponding author: Juliana Milani Araujo
 Department of Veterinary Medicine, Federal University of Viçosa – UFV,
 Av. PH Rolfs, s/n, Campus Universitário, CEP 36570 000, Viçosa, MG, Brazil
 e-mail: milanibio@yahoo.com.br

infection between the ages of 15 and 23 weeks (SOULSBY, 1982), animals older than six months may still acquire the infection, such that 30% of them are infected (WELLS et al., 1998). Helminthic parasitism in equines, as well as in other domestic animals, is combated through using anthelmintics. However, these drugs may not present a satisfactory effect, given that parasitic resistance is already widespread around the world (BRAGA et al., 2009).

Thus, integrated control of helminthoses can be seen as a new approach, since biological control can be used synergistically with chemical control (BRAGA et al., 2009). Biological control is centered on using nematophagous fungi that can act as predators, ovicidal agents and endoparasites. These fungi, which are mainly predators, have been shown to be effective for reducing nematode populations, both in laboratories and under field conditions. *Duddingtonia flagrans* and *Monacrosporium thaumasium* are predators and have been used worldwide to combat the infective larvae of nematode parasites of productive domestic animals (PAZ-SILVA et al., 2011; BRAGA et al., 2011).

The objective of the present study was to evaluate the control of infective *Strongyloides westeri* larvae (L_3) by nematophagous fungi after gastrointestinal transit in donkeys (*Equus asinus*).

To obtain *S. westeri* L_3 , fecal cultures were performed on positive feces from naturally infected young donkeys. Next, these larvae were classified in accordance with the criteria established by Soulsby (1982). In order to induce formation of fungal mycelia of *D. flagrans* (AC001) and *M. thaumasium* (NF34), culture discs of approximately 4 mm in diameter, in 2% water-agar, were transferred to 250 mL Erlenmeyer flasks containing 150 mL of liquid YPG medium (glucose, peptone and sodium yeast extract), and were kept under continual stirring at 120 rpm in the dark and at a temperature of 26 °C for 10 days. After this period, the mycelia were removed, filtered and weighed on an analytical balance.

In the *in vivo* assay, 12 female donkeys were used, with a mean weight of 240 kg. They had previously been dewormed by means of vermifuge for equines at an oral dose of 200 µg/kg live weight of 1% ivermectin and 6.6 mg/kg live weight of pyrantel pamoate (Centurion Vallé®, Montes Claros, Minas Gerais, Brazil). This was done 14 days before they received pellets containing a mycelial mass of isolates of *D. flagrans* (AC001) or *M. thaumasium* (NF34), or pellets without fungus (control).

The animals were separated into three groups (one group treated with the isolated AC001, one group treated with the isolate NF34 and a control group), with four animals in each group. In the groups treated with AC001 and NF34, each animal received 100 g of pellets in a single dose containing a mycelial mass of fungus. For this, each of the fungi was mixed into 100 g of commercial feed for horses. The control group received a single administration of 100 g of pellets without fungus.

After administration of the fungi, fecal samples were collected from the animal groups at different times (after 12, 24, 48 and 72 hours). These samples were then homogenized, and 4 g aliquots of feces were placed in Petri dishes of 9 cm in diameter, containing 2% water-agar (2% WA). These dishes were placed in an incubator at 25 °C, in the dark. Each Petri dish of the groups tested (treated and control groups) contained 1,000 L_3 of *S. westeri*. At each collection time, five repetitions were performed. To prove that the fungi tested actually passed through the gastrointestinal tract,

and to identify them, the classification keys for fungal structures (conidia and/or chlamydospores) proposed by Van Oorschot (1985) and Liu and Zhang (1994) were used. The Petri dishes were viewed every day. Subsequently, on the fifteenth day, the L_3 that had not been destroyed by the fungus were recovered from the Petri dishes by means of the Baermann technique. The data obtained were subjected to analysis of variance (F test) and then regression analysis. The means were compared using the Tukey test at the 1% level of probability.

It was observed that the fungi *D. flagrans* (AC001) and *M. thaumasium* (NF34) destroyed the *S. westeri* L_3 , after passage through the gastrointestinal tract of the donkeys. At each of the collection times studied, the following percentage reductions were found: 81.2% and 81.1% (12 hours); 62.7% and 87.2% (24 hours); 78.6% and 76.7% (48 hours); and 85.3% and 92.2% (72 hours), for the isolates AC001 and NF34, respectively, in comparison with the control group (Figure 1). At the collection time of 72 hours, both isolates (AC001 and NF34) showed higher predatory activity and, consequently higher percentage reductions in the L_3 recovered from *S. westeri*.

On the other hand, the coefficients of the linear regression curves for *S. westeri* L_3 recovered from Petri dishes, relating to the collections as a function of time were: 5.54 for the controls, -1.25 for *D. flagrans* (AC001) and -1.06 for *M. thaumasium* (NF34). In the feces of the treated groups, conidia and chlamydospores were identified according to the fungal species tested (*D. flagrans* and *M. thaumasium*).

Araújo et al. (2004) mentioned that using nematophagous fungi for biological control of gastrointestinal parasites of domestic animals may reduce the soil contamination by acting directly on the infective larvae present in the environment. On the other hand, although horses and donkeys generally harbor the same genera of gastrointestinal helminthic parasites, there are no studies with enough data regarding strategic control of helminths in donkeys. In this context, Araujo et al. (2010) reported that three fungal genera (*Arthrobotrys*, *Duddingtonia* and *Monacrosporium*) were

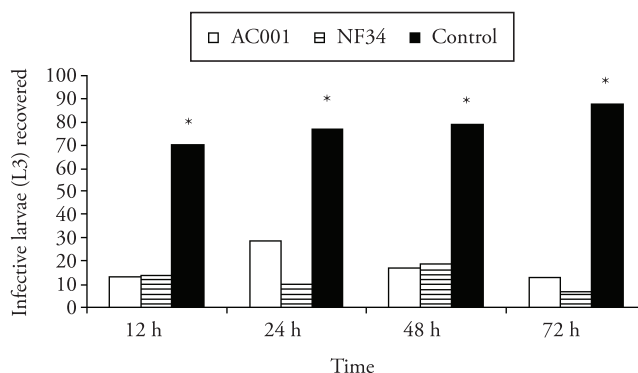


Figure 1. Mean numbers of infective larvae (L_3) of *Strongyloides westeri* that were recovered from 2% water-agar medium by means of the Baermann method on the fifteenth day of treatment with the fungal isolates *Duddingtonia flagrans* (AC001) and *Monacrosporium thaumasium* (NF34), and in the control group (without fungus). Asterisk denotes difference ($p < 0.01$) between the isolates tested and the control group at the times studied.

efficient at destroying *S. westeri* L₃ in an *in vitro* assay. In this paper, the results showed that there was no difference ($p > 0.05$) in predatory activity between the fungi tested and therefore either of the isolates could be used in *in vivo* tests. This premise provided the justification for conducting the present study, and showed the need for knowledge regarding alternative approaches towards control of helminth parasites in donkeys. In addition, Araujo et al. (2010) used horses for the *in vivo* test, thus showing that the fungi *D. flagrans* and *M. thaumasium* have also been effective in passing through the gastrointestinal tract of these animals. Nonetheless, despite being in the same group of animals (equines), donkeys are a different species and thus, studies that can demonstrate alternative control methods for endoparasites, in particular in relation to *S. westeri*, are important.

Several studies have been conducted with regard to biological control of nematode parasites of horses, both *in vitro* and under natural conditions, which once again denotes the need to extrapolate these studies to other species of equines, such as donkeys. Tavela et al. (2011) studied the fungus *M. thaumasium* (NF34) in the field, administered to horses, and demonstrated that it was effective on the larvae of cyathostomins through decreasing the recurrence of helminth infections. Similar results, thereby confirming the action of this fungus, were also found in the present work, which proved that this isolate remained viable after passage through the gastrointestinal tract of donkeys.

The efficacy of the fungus *D. flagrans* (AC001) was also demonstrated in a study by Braga et al. (2009) that consisted of a six-month field test using weekly doses of pellets containing this fungus, among horses. A difference in parasite loads ($p < 0.05$) was recorded between the animals in the treated group and those in the control group. This result is also in accordance with the findings from the present study relating to the action of the isolate AC001 on *S. westeri* L₃.

The regression curve results demonstrated that the two fungal isolates tested had a negative linear correlation coefficient. This inverse correlation (negative) between the variables proved that the fungal isolates continued to have a viable predatory capacity after passage through the gastrointestinal tract of these domestic animals. This information is in agreement with the study on horses by Assis and Araujo (2003), who found a regression curve with a negative value.

The results presented here justify the need to conduct studies in the field, with longer intervals, in order to observe the efficiency of the fungus *D. flagrans*, or even *M. thaumasium*, for environmental control of nematodes in donkeys, which may contribute towards a better and more integrated approach to control of helminths in this species of domestic animal.

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