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Biological control of horse cyathostomin (Nematoda: Cyathostominae) using the nematophagous fungus *Duddingtonia flagrans* in tropical southeastern Brazil

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ABSTRACT

The viability of a fungal formulation using the nematode-trapping fungus *Duddingtonia flagrans* was assessed for the biological control of horse cyathostomin. Two groups (fungus-treated and control without fungus treatment), consisting of eight crossbred mares (3–18 years of age) were fed on *Cynodon* sp. pasture naturally infected with equine cyathostome larvae. Each animal of the treated group received oral doses of sodium alginate mycelial pellets (1 g/(10 kg live weight week)), during 6 months. Significant reduction ($p < 0.01$) in the number of eggs per gram of feces and coprocultures was found for animals of the fungus-treated group compared with the control group. There was difference ($p < 0.01$) of 78.5% reduction in herbage samples collected up to (0–20 cm) between the fungus-treated group and the control group, during the experimental period (May–October). Difference of 82.5% ($p < 0.01$) was found between the fungus-treated group and the control group in the sampling distance (20–40 cm) from fecal pats. During the last 3 months of the experimental period (August, September and October), fungus-treated mares had significant weight gain ($p < 0.01$) compared with the control group, an increment of 38 kg. The treatment with sodium alginate pellets containing the nematode-trapping fungus *D. flagrans* reduced cyathostomin in tropical southeastern Brazil and could be an effective tool for biological control of this parasitic nematode in horses.

1. Introduction

A large variety of helminths are known to parasite horses. Nematodes, mainly cyathostomin species, are the most common and important among them. Also known as small strongyles, cyathostomin infections are responsible for causing anemia, weight loss, intestinal colic, and death in horses (Assis and Araújo, 2003). They are the most prevalent parasites in horses, present throughout the year in the pasture, with a wide distribution in different age groups (Barbosa et al., 2001; Quinelato et al., 2008).

Klei and Chapman (1999) reported field data suggesting that horses can acquire resistance to helminths with age, which is confirmed by the reduced parasite load and egg count in feces. This response is slow and inconsistent in most animals and unrelated to the intensity of previous contact with parasite.

Kaplan (2002) and Matthews et al. (2004) discussed that worm control in horses is usually carried out with

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anthelmintic drugs, which have not been totally effective for the control of these nematodes since their action is restricted to adult parasites and there is occurrence of resistance.

The continued use of the same anthelmintic class, as well as the rapid rotation of compound groups, introduction of resistant worms and the use of doses lower than the recommendation should be avoided (Mota et al., 2003). Biological control using natural nematode antagonistic fungi is among the most viable alternatives. These organisms comprise different types of fungi classified into predators, endoparasites and opportunists, whose action is concentrated in the fecal environment and directed against free-living parasitic larvae. Within the predator group, the species *Duddingtonia flagrans* stands out as the most promising for the control of gastrointestinal nematodiasis in domestic animals (Terrill et al., 2004; Dias et al., 2007a). However, to be used as a biological control agent, nematophagous fungi must have ability for nematode capture and survive passage through gastrointestinal tract (Waller et al., 1994).

Sodium alginate-based formulations containing *D. flagrans* mycelial mass have been experimentally evaluated against parasitic nematodes of animals in laboratory and field conditions (Araújo and Sampaio, 2000; Araújo et al., 2000; Dias et al., 2007b), but none these formulations have been developed for the control of parasitic nematodes of horses in the field.

The objective of the present study was to test an alginate pellet formulation containing *D. flagrans* for the biological control of cyathostomin in horses raised in fields.

2. Materials and methods

2.1. Fungal cultures

Isolate (AC001) of *D. flagrans*, a nematode-trapping fungus belonging to the genus *Duddingtonia*, was kept in test tubes at 4 °C containing 2% corn-meal-agar (2% CMA) in the dark. The isolated was obtained from a Brazilian soil using the soil sprinkling method (Duddington, 1955), modified by Santos et al. (1991).

Fungal mycelia were obtained by transferring culture disks (approximately 5 mm in diameter) of fungal isolates in 2% CMA to 250 mL Erlenmeyer flasks with 150 mL liquid potato-dextrose medium (Difco), pH 6.5, and incubated under agitation (120 rpm), in the dark at 26 °C, for 10 days. Mycelia were then removed for pelletizing using sodium alginate as described by Walker and Connick (1983) and modified by Lackey et al. (1993).

2.2. In vivo experimental assay

The experiment was conducted at the horse experimental sector of the Federal University of Viçosa, Viçosa, MG, Brazil, latitude 20°45′20″S, longitude 42°52′40″W, from May to October 2007.

In the beginning of the experiment, the 3–18 year old crossbred mares were previously dewormed with 200 μg/kg live weight Ivermectin 1% and 6.6 mg/kg live weight Pyrantel Pamoate (Centurion Vallé®, Montes Claros-Minas Gerais, Brazil).

Fourteen days after the anthelmintic treatment, the mares were separated into two groups (fungus-treated and control) of eight animals each on the basis of age and weight. Mean age and mean weight of the fungus-treated group were 6.3 (±6.1) and 386.2 (±54.07) respectively, and 7.1 (±4.7) and 381.1 (±53.91) of the control group respectively. Mares were allocated to two 2.5 ha paddocks of *Cynodon* sp., that had been previously grazed by young and adult horses and were naturally infested with equine cyathostomin larvae. Then, each animal of the treated group received twice a week 1 g pellets/10 kg live weight, containing *D. flagrans* mycelial mass combined with 100 g of horse commercial ration, as described by Assis and Araújo (2003). The treatment was offered during 6 months starting from May 2007. Animals of the control group received 1 g pellets/10 kg live weight without fungus. From the beginning (May) to the end (October) of the experiment, animals from both groups were monthly weighed. During the experiment, mares were fed daily with 2 kg of commercial ration with 14% soybean meal, 83.1% corn meal, 14.5% salt, 1.5% limestone and 14% protein.

After the mares had been moved to the paddocks, samples of fresh feces were collected once a week directly from the rectum, 72 h after the treatment, to determine egg per gram of feces (EPG), according to Gordon and Whitlock (1939) and modified by Lima (1989).

Coprocultures were established together with EPG counts; 20 g of feces were mixed with ground, moistened and autoclaved industrial vermiculite (NS Barbosa Ind. Com.®) and taken to an oven at 26 °C, for 8 days, to obtain cyathostome larvae. Larvae were identified to the genus level as described by Bevilaqua et al. (1993). EPG and larvae recovered from coprocultures of animals of both treated and control groups were recorded and percentage of larval reduction was determined according to Mendoza-De-Guives et al. (1999):

$$\text{reduction}\,(\%) = \frac{\text{mean L}_3\text{ recovered from control group} - \text{mean L}_3\text{ recovered from treated group}}{\text{mean L}_3\text{ recovered from control group}} \times 100$$

Every 15 days, two herbage samples were collected from both the treated and control groups, from each paddock, in a zigzag pattern from several and alternated points, 0–20 and 20–40 cm away from fecal pats, in each paddock of the different groups, according to Amarante et al. (1996). Herbage samples were always collected in the morning at 8 a.m. Then, a 500 g herbage sample was weighed, and parasitic nematode larvae were recovered following the procedure of Lima (1989). The samples were incubated in a drying oven at 100 °C, for 3 days, to determine dry matter. Data were transformed into larvae per kg of dry matter.

Climate data referring to averages of maximum, average and minimum monthly temperatures, air relative humidity and monthly rainfall were daily recorded in a meteorological station in the area.

The egg count curves (EPG) originated from the coprocultures, number of infective larvae recovered from paddocks (L_3), correlation between EPG and recovered L_3