Experimental Parasitology 194 (2018) 9–15

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

Reduction of bovine strongilides in naturally contaminated pastures in the southeast region of Brazil

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ARTICLE INFO

Keywords:
Nematophagous fungus
Arthrobotrys cladodes
Biological control
Nematode

ABSTRACT

Biological control through the use of nematophagous fungi is a sustainable alternative for combatting helminthes in domestic animals and allows a reduction in the use of anthelmintics. The objective of this research was to evaluate the efficacy of the *Arthrobotrys cladodes* var *macroides* fungus in a pelleted formulation, based on sodium alginate and administered twice a week orally, as an alternative for the biological control of nematodes in field-grown young cattle. The experiment was conducted in a farm located in the municipality of Viçosa, MG, where 12 cattle, seven to nine months old, were allocated in two groups (treated group and control group) and distributed in pickets of *Brachiaria decumbens*, naturally infested with nematode larvae. The animals in the treated group received 1g of sodium alginate matrix pellets for every 10 kg of animal live weight, containing the nematophagous fungus *Arthrobotrys cladodes* var *macroides* and administered twice a week in conjunction with commercial feed. In the control group, each animal received 1 g of pellets for every 10 kg of animal live weight, without fungal mycelium added to the feed. Samples of feces and pastures were collected fortnightly for 12 months. The results showed that the most prevalent nematode genera in the coprocultures were *Haemonchus* sp., *Cooperia* sp. and *Oesophagostomum* sp., reflecting the results found in forage. The pasture that contained the animals that received feed with the fungus presented a reduction of 59% and 52% of larvae recovered at distances of 20 cm and 40 cm from the fecal pats, respectively. The mean number of eggs per gram of feces each month and animal body weight did not differ ($p > 0.05$) between the treated and control groups. Stool and soil samples from both groups were colonized by *A. cladodes* fungus and other fungi. Administration of *Arthrobotrys cladodes* var *macroides* mycelium by means of a sodium alginate matrix twice weekly reduced larval infestation of the surrounding pasture, indicating that this fungus may be a promising biological control of infecting forms of nematodes present in the environment.

1. Introduction

To cater for the demand of a growing population and, in particular, current customer demands placed on the animal production chain (Charlie et al., 2014), the main challenge in the coming years for industry and livestock production in general will be the production of high quality food in an ethical, environmentally acceptable manner and one that maintains economic viability (Verschave et al., 2016).

This growing demand for a more extensive exploitation of cattle rearing causes an increase in pasture stocking (Claudino, 2014), which leads to an increase in health problems (Venturini and Menezes, 2016).

Helminth infections comprise an important group of diseases in grazing ruminants (Charlie et al., 2014; Grisi et al., 2014) and the profitability of livestock-related activities can be significantly reduced by these parasites (Grisi et al., 2014).

The future control of these parasites, however, is challenged by several factors, such as the development of anthelmintic resistance, and changes in climate and agricultural management (Verschave et al., 2016; Silva et al., 2016).

The combined use of chemical and biological control methods can be a viable strategy for livestock health, cost reduction, low resistance and toxicity to the chemical bases used, as well as reducing chemical residues from animal products into the environment (Soares and Monteiro, 2011). In this perspective the biological control involving the

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https://doi.org/10.1016/j.exppara.2018.09.008
Received 7 June 2018; Received in revised form 21 August 2018; Accepted 16 September 2018
Available online 18 September 2018
0014-4894/

use of nematophagous fungi has been field tested with very interesting results (Assis et al., 2013, 2015). These fungi break the life cycle of the nematode by capturing infective larval phases before their migration from fecal matter to pasture, where they would otherwise be consumed by grazing animals (Braga and Araújo, 2014).

Arthrobotrys cladodes var. *macroides* shows high production of conidia and produces chlamydospores. According to Anan'ko and Teplyakova (2011) and Silva et al. (2015), these characteristics are desirable when selecting a nematophagous fungus, since genera that have high production of conidia and chlamydospores have the advantage of dispersal and colonisation of the environment. Consequently, these fungi would be more successful than other genera in the control of nematodes.

In view of these premises, the effectiveness of the *Arthrobotrys cladodes* var *macroides* as an alternative means of biological control of nematodes in young field reared cattle was evaluated, administered orally twice a week in a pelleted formulation of sodium alginate.

2. Material and methods

2.1. Fungus

The nematophagous fungus, *Arthrobotrys cladodes* var *macroides* (CG isolate 719), was obtained from the Laboratory of Parasitology of the Veterinary Department of the Federal University of Viçosa, Minas Gerais, where it was kept at 4 °C under a light in test tubes containing corn meal agar (CMA; 2%). The fungus was inoculated in a Petri dishes (9 cm diameter) containing 2% water agar medium (WA). The fungus was allowed to grow for seven days. For induction of fungal mycelium formation, approximately 5 mm agar fragments containing mycelium and fungal spores were transferred to 250 ml Erlenmeyer flasks containing 150 ml of GPY liquid medium (glucose, soy peptone and yeast extract) and pH 6,5. Contents were stirred at 120 rpm, in the dark conditions at 26 °C for 21 days. After this period, the mycelium was removed for the manufacture of the pellets, which were incorporated into a matrix of sodium alginate according to the technique described by Walker and Connick (1983) modified by Lackey et al. (1993). The pellets used in the experiment were oven-dried and maintained at a moisture content of 30%.

2.2. In vivo experiment

The experiment was conducted at a farm located in the municipality of Viçosa, in the state of Minas Gerais, southeastern Brazil (latitude 20° 45 ′20 “S, longitude 42° 52′ 40" W).

Twelve bovine animals, seven to nine months old Dutch-Zebu mestizos, weighing 180 kg on average, were pretreated with 1% ivermectin anti-helmintic at a dose of 1 ml/50 kg live weight and albendazole suspension at a dose of 1 ml/20 kg live weight. Twenty-one days after anthelmintic treatment and confirmed absence of nematode eggs in the feces, animals were randomly divided into two groups of six animals. Each group was distributed in two 2-ha pickets with a population of the grass *Brachiaria decumbens* naturally infested with helminth larvae due to a previous history of grazing by young and adult animals.

In the first group, each animal was treated with 1 g of pellets for each 10 kg of animal live weight containing the *Arthrobotrys cladodes* fungus. The pellets were administered twice a week together with commercial feed (15% crude protein, 78.8% maize, 20.7% soybean meal, 0.17% bicalcium phosphate, 0.03% sulfur, 0.03% microminerals, 0.03% vitamins, 0.15% common salt, 0.01% limestone) with a dry matter content of 88%. In the second (control) group, each animal received 1 g of pellet for every 10 kg of animal live weight, without fungal mycelium added to the feed.

The experiment lasted for 12 months, during which only samples of fecal material from the animals and pasture samples were collected. All the proposed methodology was in accordance with the guidelines of the World Association for the Advancement of Veterinary Parasitology (WAAVP), following methods for evaluating the efficacy of anthelmintics in ruminants: cattle and sheep described by Powers et al. (1982) and second edition of the guidelines for evaluating the efficacy of anthelmintics in ruminants: cattle, sheep and goats cited by Wood et al. (1995). This trial rigorously followed all the procedures recommended by the standards of conduct for the use of animals and certified by the Commission of Ethics in the Use of Animals (CEUA/UFV) by case nº 21/2016.

2.3. Fecal material harvesting and processing

Every 15 days, after introduction of the animals into the pastures, fecal samples from all animals in each group were collected directly from the rectal ampulla. In these samples, egg counts per gram of feces (EPG) were determined using the method of Gordon and Whithlock (1939), modified by Lima (1989).

Every 15 days, coprocultures were produced with 20 g of feces mixed with vermiculite and incubated at 26 °C for 15 days to obtain infective larvae. The larvae were later identified according to Keith (1953).

2.4. Pasture samples

Every 15 days, two pasture samples (20 cm and 40 cm distance from the fecal pats) were collected from the pickets of the treated and control groups in a zigzag pattern from six alternated points, according to Raynaud and Gruner (1982). Samples of pasture (500 g) were used to recover infective larvae (L3) following the methodology described by Lima (1989). Subsequently, the larvae were examined under an optical microscope, counted and identified according to the criteria established by Keith (1953).

The 500 g samples of pasture that were used to recovered the larvae were placed in an oven at 100 °C to obtain dry matter. The data obtained were transformed into the number of larvae per kilogram of dry matter.

2.5. Animal weight gain

The weight of the animals was recorded monthly.

2.6. Weather data

Climatic data on average minimum and maximum monthly temperatures and monthly precipitation were recorded daily at a specialized meteorological station at the Campus of the Federal University of Viçosa, MG, Brazil.

2.7. Isolation and identification of nematophagous fungi

Soil and fecal samples for both groups were collected in July 2016 and January 2017. Each picket was divided into four quadrants and samples were collected randomly. The soil samples were homogenized and processed in triplicate using the Duddington (1950) soil-scattering technique, modified by Santos (1991). This involved placing 2 g of soil sample in the centre of a Petri dish containing agar-water culture medium (2%). The same procedure was performed with the stool samples. Free-living nematodes, *Panagrellus* sp., were used to stimulate the development of predatory structures by nematophagous fungi. The seeded plates were incubated in a BOD germ chamber at 25 °C. The resulting plaques were visualized weekly for a period of two months. In identifying the entrapment of nematodes in the Petri dish, sample of the fungus was collected with a platinum loop and transferred to a Petri dish containing 2% corn meal agar (CMA) culture medium with *Panagrellus* sp. and incubated in a BOD germ chamber at 25 °C for