Association between Duddingtonia flagrans, Dimethylsulfoxide and Ivermectin for the control of Rhabditis spp. in cattle

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Abstract

Cattle parasitic otitis caused by the nematode Rhabditis spp. is a serious health problem in Brazil, since it does not have an effective control. In vitro studies associating biological control and chemical control as an alternative method showed promising results. The objective of the present study was to evaluate the combined use of the fungus Duddingtonia flagrans (AC001), 10% dimethylsulfoxide and 1.87% Ivermectin for the in vivo control of Rhabditis spp., in naturally infected Gyr cattle. For this purpose, 48 animals, whose infection in both ears was diagnosed, were randomly assigned to 6 groups: Group 1 (Ivermectin 1.87%); Group 2 (10% dimethylsulfoxide); Group 3 (AC001); Group 4 (Ivermectin 1.87% + dimethylsulfoxide 10%); Group 5 (1.87% Ivermectin + AC001); Group 6 (10% dimethylsulfoxide + AC001). The treatments were performed in a single dose, in the right ears, with the left ears remaining untreated, as a control group. There was a significant reduction (p<0.01) in the recovery of nematodes in the treated groups in relation to the control, with the following best efficacies: Groups 1 and 2, 47% and 52.9%, respectively, seven days after treatment; Groups 3, 4 and 5, 47.8%, 48.6% and 36.7%, respectively, 14 days post-treatment; Group 6, 38.4%, 21 days post-treatment. It was concluded that the combination of chemical compounds and D. flagrans in a single application were effective for the in vivo control of Rhabditis spp. in naturally infected cattle.

Full Text

The nematode *Rhabditis* spp. causes parasitic otitis in cattle, mainly in Gyr and Indubrasil breeds and also in their crossbreds (Leite et al., 2013). Thus, attention is drawn to the ideal racial pattern for crossbreeding, delivering dual-purpose animals to the producer. It is regretted, however, that the initial genetic improvement targeted racial traits, especially head and ear, which consequently resulted in an auricular pavilion favorable to parasite development and ear infections (Vieira et al., 2001).

Rhabditis spp. transmission is facilitated in the presence of dipterans (flies), leading to infections predominantly in both ears, which may progress to abundant purulent secretion with a large number of parasites (Vieira et al., 2001; Verocai et al., 2007).

Specifically in relation to treatments used in parasitic otitis, there are many drugs that have been used, however, the results are unsatisfactory and the recurrence of infections is a routine problem in animals (Verocai et al. 2007; Barbosa et al., 2016). Thus, alternatives that may be synergistic to control with anthelmintic drugs have been researched. Recently, Sobral et al. (2019) demonstrated the nematophagous fungi in vitro effectiveness, including *Duddingtonia flagrans*, in the control of *Rhabditis* spp. In another recent study, Ferraz et al. (2019) report that the use of 1% dimethylsulfoxide (DMSO) and 100% mineral oil together with nematophagous fungi were effective *in vitro* in the destruction of *Rhabditis* spp.

There are no reports on the association of nematophagous fungi with chemical compounds for the *in vivo* control of *Rhabditis* spp., so, the present study aimed to test the combined action of *D. flagrans*,

DMSO and Ivermectin in natural infestations by *Rhabditis* spp. in Gyr cattle.

Visits were carried out on a farm in the municipality of Anchieta, State of Espírito Santo, Brazil, between September and November 2021. This farm had a herd of 200 cattle of the Gyr and Girolando breeds, with at least 70 animals presenting clinical signs of external otitis, such as apathy, head shaking, otorrhea, discomfort to the touch and itching.

To verify the infection by *Rhabditis* spp., seven days before beginning the experiment, the animals with signs of external otitis were restrained in a containment trunk and their head were immobilized. Subsequently, the technique of ear canal washing was performed. Fifty mililiters of saline solution was applied in both ear canals. Subsequently, the material was collected in a sterile stainless-steel tray and placed in 50 mL Falcon tubes. Then, the samples were packaged and sent for laboratory analysis. After confirming the presence of infection by *Rhabditis* spp. in both ear canals, by visualization under an optical microscope, 10x and 40x objectives, the animals were able to the experiment.

Conidia of the nematophagous fungus *D. flagrans* (AC001) were used. Also, 10% dimethyl sulfoxide (Vetnil, Brazil), 100% mineral oil (União Química, Brazil) and 1.9% ivermectin (Eqvalan® Pasta - Merial, Brasil) were used.

Six groups with eight animals each were assembled. Experimental protocols were performed only on the right ears, on Day 0 (D0): Group 1 - Ivermectin 1.9%, 500 µl/animal, topically in the ear canal; Group 2 – ear canal washing with a 10% DMSO solution (45 mL/animal); Group 3 – 12 mL solution containing $3x10^6$ *D. flagrans* conidia in mineral oil vehicle, topically in the ear canal; Group 4 - Ivermectin 1.9%, 500 µg/animal + ear canal washing with a solution of 10% DMSO (45 mL/animal) w/v, both topically in the ear canal; Group 5 - Ivermectin 1.9%, 500 µg/animal, topically in the ear canal + 12 mL solution containing $3x10^6$ *D. flagrans* conidia in mineral oil vehicle w/v; Group 6 - ear canal washing with a 10% DMSO solution (45 mL/animal) + 12 mL solution containing $3x10^6$ *D. flagrans* conidia in mineral oil vehicle v/v. In all animals, the left ears served as the control group, in which only the washing was performed on D0 with 50 mL of saline solution.

The percentage of reduction/efficacy of the experimental protocols in the studied groups, in relation to the control, was evaluated using the following formula:

$$\% \text{ Reduction} = \frac{\text{average of the control group} - \text{average of the treated group}}{\text{average of the control group}} \times 100$$

The results obtained were analyzed by means of analysis of variance (ANOVA) at a 5% probability level. The Tukey's test was applied as a post-assay test.

There was a significant reduction ($p<0.01$) in the number of nematodes in the treated groups compared to the control group (Table 1). Groups 1 and 2 showed higher reductions of nematodes seven days after treatments (D7), 47% and 52.4%, respectively. Groups 3, 4 and 5 showed higher reductions in infections

14 days after treatments (D14), with 47.8%, 48.6% and 36.7%, respectively. Group 6, on the other hand, showed higher reductions 21 days after treatments (D21), with 38.4%.

The authors emphazise the fact that the Gyr herd and its crossbreeds are important in milk production in Brazil, and therefore the parasitic otitis caused by *Rhabditis* spp. causes incalculable damage to this livestock activity (Bossi et al., 2015; Sobral et al., 2019). The literature argues that the control of parasitic otitis is difficult, due to a large variety of therapeutic protocols tested without success (Souza et al., 2008; Leite et al., 2013).

In the present study, the fungus *D. flagrans* (AC001) was applied topically in a mineral oil vehicle (Group 3), showing a maximum reduction of 47.8%, 14 days after treatment. It is important to note that this is the first report of in vivo/topical administration of *D. flagrans* in the control of *Rhabditis* spp. under natural conditions, which further reinforces the use of this fungus in parasite control. Rodrigues et al. (2021) recorded the efficacy and safety of the commercial formulation Bioverm® (*D. flagrans*) and which in the future may act in the prevention and control of parasitic otitis. This result is still in agreement with what was proposed by Ferraz et al. (2019), who associated nematophagous fungi with chemical compounds in an *in vitro* assay and obtained promising results, with a future premise of designs under natural conditions, as presented in this paper. It is noteworthy, however, that other vehicles, forms of application and complementary dosages of treatments can contribute to a better effect in the elimination of nematodes. Thus, it is important to continue with this research line.

In Groups 5 and 6, *D. flagrans* was associated with the chemical compounds, Ivermectin 1.9% and 10% DMSO, respectively, with reduction, after 21 days, of 32.6% and 38.4%. We chose to use Ivermectin 1.9% in the form of a topical paste to provide better adherence of the product to the animals' ear canal. Even so, when the anthelmintic was used alone, in Group 1, a reduction of 47% (D7) was observed, demonstrating a low effect on the elimination of nematodes. Because it is widely used as an endectocidal drug, the incorrect use of ivermectin over the decades has resulted in parasite resistance, a serious and global problem (Laing et al., 2017). Other unsatisfactory results with the use of Ivermectin 0.5% have already been reported (Verocai et al., 2009; Barbosa et al., 2016).

In Group 2, the use of a 10% DMSO solution showed a reduction of 52.4%, seven days after treatment. However, the combination of the compound with other drugs or the formulation of a paste with better fixation may be the solution for more satisfactory results in the future. Dimethylsulfoxide is an organic compound from the processing of petroleum used in the pharmaceutical industry. Studies point to various activities for this compound, such as anti-inflammatory, antioxidant, rapid permeability in tissues, analgesic, immunomodulator and substance carrier (Crivellenti et al., 2013; Picoli et al., 2015).

It was concluded that the combination of *D. flagrans*, 10% DMSO and 1.9% Ivermectin was effective for the in vivo control of *Rhabditis* spp., which is an important contribution to the control of cattle parasitic otitis.

Declarations

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Conflict of interest

The authors declare that they did not have any conflict of interest relevant to the content of this article.

Ethics approval

The activities involved in this research were approved by Ethics Committee for Animal Use (CEUA/UVV), under protocol number 608-2021.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability

Not applicable.

Authors' contributions

Samilla A. Sobral and Fabio R. Braga contributed to the study conception and design. Material preparation, data collection and analysis were performed by Samilla A. Sobral, Carolina M. Ferraz, Rômulo I. L. Souza, Luanderson M. Queiroz, Natália Reinó, Otávio L. Fidelis Junior, Fernando L. Tobias and José A. L. Correia. The first draft of the manuscript was written by Samilla A. Sobral, Jackson V. Araújo, Vinícius L. R. Vilela, Filipe E. F. Soares and Fabio R. Braga; and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Table

Table 1. Averages and percentages of reduction – R(%) of the nematode *Rhabditis* spp., recovered in the groups of treated animals: Group 1 - Ivermectin 1.9%, 500 µg/animal, topically in the ear canal; Group 2 – ear canal washing with a 10% DMSO solution (45 mL/animal); Group 3 – 12 mL solution containing $3x10^6$ *D. flagrans* conidia in mineral oil vehicle, topically in the ear canal; Group 4 - Ivermectin 1.9%, 500 µg/animal, topically in the ear canal + ear canal washing with a solution of DMSO 10% (45 mL/animal) w/v; Group 5 - Ivermectin 1.9%, 500 µg/animal, topically in the ear canal + 12 mL solution containing $3x10^6$ *D. flagrans* conidia in mineral oil vehicle w/v; Group 6 - ear canal washing with a 10% DMSO solution (45 mL/animal) + 12 mL solution containing $3x10^6$ *D. flagrans* conidia in mineral oil vehicle v/v; over days 7 (D7), 14 (D14) and 21 (D21), in relation to their respective control groups before treatment, at day zero (D0).

Groups	Days after treatment						
	D0 (control)	D7		D14		D21	
	Average	Average	R(%)	Average	R(%)	Average	R(%)
Group 1	145^a	77^b	47	91^b	37.2	98^b	32.4
Group 2	145^a	69^c	52.4	67^c	53.8	85^{bc}	41.4
Group 3	232^a	140^b	39.7	121^b	47.8	140^b	39.7
Group 4	107^a	68^b	36.4	55^b	48.6	71^{ab}	33.6
Group 5	632^a	404^b	36	400^b	36.7	426^b	32.6
Group 6	479^a	358^b	25.4	320^{bc}	33.2	295^{bc}	38.4

Averages followed by the same lowercase letter in the lines do not differ statistically ($p \geq 0.01$) by Tukey's Test.