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Combined use of chemical and biological compounds to control hookworm

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Abstract

The aim of this study was to evaluate the combined use of different chemical (albendazole, ivermectin, glycerine and Vaseline) and biological (*Monacrosporium thaumasium*) compounds in the control of *Ancylostoma caninum*. Infective larvae of *A. caninum* were obtained from coprocultures of positive faeces from naturally infected dogs. We used 1% ivermectin, 1% albendazole, 100% glycerine, 100% Vaseline and an isolate of the nematophagous fungus *M. thaumasium* (NF34), alone or in combinations. Next, an experimental test was set up with 16 groups in microtubes, with a 24-h interaction. The groups (G1 to G15) that contained any chemical or biological compound (NF34) and/or their combined use (chemical + biological) showed a difference in relation to the control group, except G5 – Vaseline 100% without combinations. It was concluded that, even on an experimental basis, the combined use of anthelmintic drugs with biological control was efficient; however, more studies must be carried out in order to elucidate the synergistic action between chemical and biological compounds to be used in the effective control of hookworms in the future.

Introduction

Hookworm infection is a neglected tropical disease caused by nematodes *Ancylostoma* spp., and it assumes in dogs its most pathogenic role. In terms of public health, this nematode also plays an important role in the aetiology of cutaneous larva migrans (CLM), due to the presence of infective larvae (L_3) in the soil, and its evolutionary cycle ends up accidentally occurring in humans (Jourdan *et al*., 2018). Globally, it is estimated that 440 million people are infected with hookworms, and the overall damage is estimated to be as high as $139 billion annually (Hu *et al*., 2002). In Brazil, the literature is quite vast when it comes to CLM; in practically the entire national territory, the rates are alarming and require not only parasitic but also effective public control measures (Carvalho *et al*., 2011).

There is already evidence of resistance to anthelmintics in canine hookworm populations (Kopp *et al*., 2007; Sunderkötter *et al*., 2014). On the other hand, the importance of knowing *Ancylostoma caninum* epidemiology, and not only treating infections, should be highlighted. In CLM treatment, effective anthelmintic drugs such as ivermectin and albendazole are administered orally (Veraldi *et al*., 2017; Gao & Liu, 2019). In this sense, Veraldi *et al*. (2012) evaluated and proved, in a retrospective study, the efficacy and tolerability of oral albendazole in patients with CLM. On the other hand, Fischer & Nenoff (2016) demonstrated, for the first time, the topical use of ivermectin in the treatment of a patient with CLM. However, data on the use of topical ivermectin in the treatment of CLM require further studies (Gelmetti *et al*., 2019).

In this sense, the search for collaborative measures against L_3 may prove to be an interesting strategy (Carvalho *et al*., 2011; Ferraz *et al*., 2019). In topical treatments, ointments, gels and creams are used, above all, as vehicles to help facilitate drugs and other substances through the epidermis. Examples of these vehicles include glycerine and Vaseline, which are widely used in industry. In this way, studies aimed at the combined use of acknowledged treatments (Sunderkötter *et al*., 2014), as well as effective biological control (Carvalho *et al*., 2011) of *A. caninum* L_3, may in the future fight the cause of CLM, and thereby reduce the problem of this infection.

The present study aimed to evaluate the combined use of ivermectin, albendazole, glycerine and Vaseline with the nematophagous fungus *Monacrosporium thaumasium* (NF34) on L_3 of *A. caninum*.

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Table 1. Experimental groups (G1 to G16) designed to evaluate the combined use of albendazole 1%, ivermectin 1%, glycerine 100%, Vaseline 100% and conidia of *Monacrosporium thaumasium* (NF34) against *Ancylostoma caninum* infective larvae.

Group	Experimental design
G1	120 *A. caninum* L_3/30 µl + 120 NF34 conidia/30 µl
G2	120 *A. caninum* L_3/30 µl + 10 µL albendazole 1%
G3	120 *A. caninum* L_3/30 µl + 10 µL ivermectin 1%
G4	120 *A. caninum* L_3/30 µl + 160 µL glycerine 100%
G5	120 *A. caninum* L_3/30 µl + 160 µL Vaseline 100%
G6	120 *A. caninum* L_3/30 µl + 10 µL albendazole 1% + 120 NF34 conidia/30 µl
G7	120 *A. caninum* L_3/30 µl + 10 µL ivermectin 1% + 120 NF34 conidia/30 µl
G8	120 *A. caninum* L_3/30 µl + 160 µL glycerine 100% + 120 NF34 conidia/30 µl
G9	120 *A. caninum* L_3/30 µl + 160 µL Vaseline 100% + 120 NF34 conidia/30 µl
G10	120 *A. caninum* L_3/30 µl + 10 µL albendazole 1% + 10 µL ivermectin 1%
G11	120 *A. caninum* L_3/30 µl + 10 µL albendazole 1% + 160 µL glycerine 100%
G12	120 *A. caninum* L_3/30 µl + 10 µL albendazole 1% + 160 µL Vaseline 100%
G13	120 *A. caninum* L_3/30 µl + 160 µL Vaseline 100% + 160 µL glycerine 100%
G14	120 *A. caninum* L_3/30 µl + 10 µL ivermectin 1% + 160 µL Vaseline 100% + 160 µL glycerine 100%
G15	120 *A. caninum* L_3/30 µl + 10 µL ivermectin 1% + 160 µL Vaseline 100%
G16	120 *A. caninum* L_3/30 µl + 160 µL H_2O

Material and methods

Obtaining A. caninum larvae

The third-stage larvae of *A. caninum* were obtained from fresh faeces of naturally infected dogs; to process the faeces, the Willis test was performed to find positive samples. Subsequently, coprocultures were prepared and then kept in an incubation chamber for ten days at 26°C. After this period, the larvae were extracted by the Baermann technique and identified as *A. caninum* according to the criteria described by Taylor *et al.* (2016).

Treatment

In the present study, 1% ivermectin (Merial, Brazil), 1% albendazole (Vetnil, Brazil), 100% glycerine (Dinâmica, Brazil) and 100% Vaseline (Chemical Union, Brazil) were used. These compounds have been used in topical treatment protocols against CLM and were obtained from local stores.

In addition to these compounds, a nematophagous fungus isolate was used: *M. thaumasium* (NF34), which is present in Brazilian soil and came from the Parasitology Laboratory of Universidade Federal de Viçosa, Brazil. The isolate was cultured in 9-cm-diameter Petri dishes containing 2% potato–dextrose–agar culture medium. Mycelial growth across the plate was observed after seven days of culture. To obtain a conidia solution of NF34, 5 ml of distilled water was added to each Petri dish and, with the aid of a spatula, the conidia and mycelial fragments were poured into 15 ml Falcon tubes (Ferraz *et al.*, 2019).

Experimental test

Sixteen experimental groups were formed in microtubes, each one having five replicates. The numbers of *A. caninum* L_3 and conidia used in the groups were standardized using aliquots maintaining concentrations of the following: 120 nematodes; 10 µl albendazole; 10 µl ivermectin; 160 µl glycerine; 160 µl Vaseline; and 120 µl conidia. The experimental groups are presented in table 1. After 24 h of L_3 interaction with ivermectin, albendazole, glycerine, Vaseline and NF34, the content of all microtubes in groups G1 to G16 was read using 10× light microscopy, and the number of living L_3 was counted (Ferraz *et al.*, 2019).

Statistical analysis

The results obtained were evaluated by means of analysis of variance and Tukey post-test at a 1% level, using the BioEstat 5.0 software (Ayres *et al.*, 2003). The reduction percentage was calculated using the following equation: % reduction = average of living L_3 recovered in the treated group/average of living L_3 recovered in the control group × 100 (Mendoza-De Gives & Vazquez-Prats, 1994).

Results and discussion

In the present work, the action of chemical (albendazole, ivermectin, glycerine and Vaseline) and biological (NF34) compounds on *A. caninum* L_3 after a period of 24 h was verified (table 2).

As previously described, the effective environmental control of *A. caninum* L_3 still needs further research (Carvalho *et al.*, 2011). Additionally, the chemical treatment recommended for infection caused by L_3 in human skin (CLM) is based on the use of oral anthelmintic drugs; although effective, it may present some side effects, contraindication by age and even drug resistance and/or ineffectiveness (Veraldi *et al.*, 2017).

Thus, in this work, one of the objectives was to evaluate the combined use of anthelmintic drugs (chemical control) with the

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Table 2. Means, standard error and reduction percentage of *Ancylostoma caninum* larvae recovered in the experimental groups G1 to G16 after 24 h of interaction.

Group	Mean (standard error)	% reduction
G1 – NF34 + L_3	45.6 ± 5.5	46
G2 – albendazole + L_3	9.4 ± 7.0	88.4
G3 – ivermectin + L_3	15.6 ± 3.7	81.6
G4 – glycerine + L_3	76.2 ± 2.5	10.5
G5 – vaseline + L_3	81.6* ± 5.0	4.2
G6 – NF34 + albendazole + L_3	18.2 ± 4.2	78.6
G7 – NF34 + ivermectin + L_3	55.8 ± 3.0	34.5
G8 – NF34 + glycerine + L_3	29.8 ± 8.0	65
G9 – NF34 + vaseline + L_3	57.2 ± 7.3	32.8
G10 – albendazole + ivermectin + L_3	19 ± 8.4	77.6
G11 – albendazole + glycerine + L_3	22.8 ± 12.6	73.2
G12 – albendazole + vaseline + L_3	52.2 ± 4.6	38.7
G13 – glycerine + vaseline	59.2 ± 6.6	30.5
G14 – ivermectin + glycerine + vaseline + L_3	26 ± 9.0	69.4
G15 – ivermectin + vaseline + L_3	38.8 ± 4.0	54.4
G16 – H_2O + L_3	85.2* ± 4.0	–

*No statistical difference ($P > 0.05$).

fungus *M. thaumasium* (biological), glycerine and Vaseline, perhaps envisioning their combined use in the future. On the other hand, the authors recognize that there are no studies that could justify the use of a group composed of NF34 + albendazole + ivermectin + glycerine + Vaseline, since there is no report on the topical use of nematophagous fungi in humans.

Corroborating this fact, the work of Araújo & Guimarães (2002) was a pioneering study in using a solution containing NF34 fungus directly in the auditory canal of cattle infected by the nematode *Rhabditis* spp., which causes parasitic otitis. In a recent study, Ferraz *et al.* (2019) proved that the combined use of 1% dimethyl sulfoxide, 100% mineral oil and fungal conidia was efficient in controlling this nematode; however, there was already the premise of NF34 topical use applied at that time by Araújo & Guimarães (2002) only in animals. The results obtained by Ferraz *et al.* (2019) will be used in another design under field conditions.

In contrast, it was observed once again that, in the future, the use of NF34 with anthelmintic drugs might prove to be a strategy to be better studied (Ferraz *et al.*, 2019), since the literature suggests that albendazole and ivermectin have a possible fungicidal action. Vieira *et al.* (2017) mentioned that antiparasitic compounds have an *in vitro* inhibitory effect on nematophagous fungi, compromising their activity as biological control agents – something that can be observed in group G7.

The G5 group – Vaseline without combinations – showed no difference ($P > 0.01$) in L_3 reduction in relation to the control group. This fact can be explained by its hydrophobic property – that is, it practically does not dissolve in water, since the conidia/chlamydospore solution used was liquid. But, as Vaseline is used as a base in many cosmetic products, we decided to include it in this study. Furthermore, glycerine has emollient, lubricating, moisturizing and hygroscopic properties that contribute to water absorption, and, thus, there may have been a certain 'compensation' between the compounds. Either way, both glycerine and Vaseline are present in topical pharmaceutical products and have been tested here as vehicles, foreseeing their combined use in the future.

Due to the presence of drug resistance of hookworms (Kopp *et al.*, 2007) in dogs and to the worrying increase in CLM cases in the world (Alcântara *et al.*, 2019), the study of alternatives that can help in controlling the cause of this infection is justified – that is, the destruction of *A. caninum* L_3. The innovative character of this study was to cast light on the challenges of the combined use, even on an experimental basis, of anthelmintic drugs and the nematophagous fungus *M. thaumasium*, thus collaborating more and more with studies that can elucidate the synergistic action between chemical and biological control against the problems caused by hookworms.

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Conflicts of interest. None.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

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