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Title: The efficacy of predatory fungi on the control of gastrointestinal parasites in domestic and wild animals - a systematic review

Article Type: Review article

Keywords: Animals; Biological control; Duddingtonia flagrans; Gastrointestinal parasites; Predatory fungi.

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Abstract: Background: Gastrointestinal parasites like nematodes are associated with significant impacts on animal health, causing poor growth rates, diseases and even death. Traditional parasite control includes the use of anthelmintic drugs, albeit being associated with drug resistance and ecotoxicity. In the last decade, biological control of parasites using nematophagous or predatory fungi has been increasingly studied, although systematic evidence of its efficacy is still lacking. The aim of this work was to assess the evidence of efficacy of nematophagous fungi in the control of nematodes and other gastrointestinal parasites in different animal species.

Methods: Using the PICO method (Population, Intervention, Comparison and Outcomes), we performed a systematic review on the subject to search for original papers published between January 2006 and October 2019, written in English, and indexed in PubMed/Medline. Medical Subject Headings (MeSH) terms were used in the syntax. Papers were selected for detailed review based on title and abstract. Inclusion and exclusion criteria were applied, and relevant data were collected from the remaining papers.

Results: The literature search retrieved 616 papers. Eighty-nine were submitted to a detailed review. In the end, 53 papers were included in the analysis. The studies were very heterogeneous, using different fungi, doses, frequency of administration, duration of treatment, host animals, and target parasites. Considering the 53 papers, 44 studies (83% of the interventions) showed efficacy, with only 9 studies (17%) showing no significant differences when compared to control.

Conclusion: With the increasing hazards of drug resistance and ecotoxicity, biological control with predatory fungi stands out as a good tool for future parasite management, whether as a complementary treatment or as an alternative to standard parasite control.

Ms. No. Vetpar-D-20-14091R1
The efficacy of predatory fungi on the control of gastrointestinal parasites in domestic and wild animals - a systematic review

Dear Professor Michael Philipp Reichel, DrMedVet, PhD, MBA, FRCVS Co-Editor in Chief Veterinary Parasitology,

We would like to thank you and the reviewers for the comments to improve the paper.
We have performed a proof-reading of the whole manuscript.
Please find below the response to the Reviewers' comments.

Reviewer #1:
Comment: There are still numerous grammatical errors which have to be corrected.
The authors have largely addressed the previous comments of the reviewers, so with some modification, I believe the paper is worthy of publication.
Answer: Thank you for these comments. We have revised the manuscript and hope it is now suitable for publication. All the lines mentioned after reviewing, refer to the track changes version.

Comments: L 240: L1 of what? This needs to be expanded in this sentence.
L 241: This is the first mention of the species name.
Answer: These two points have been corrected (Lines 226 to 232).

Comment: L 279-280: This sentence should be removed.
Answer: The sentence has been removed (Lines 268-269).

Comment: L 292: 'ambient' temperature? 'Soil' temperature?
Answer: Thank you for noting this. It has been corrected (Line 281).

Comment: L 310: I assume this refers to efficacy?
Answer: Yes, thank you for pointing this. The sentence has been clarified (Line 297).

Comment: L 330: 'chicken's organism'?
Answer: The sentence has been corrected (Line 315).

Comment: L 337: 'translates a better overall welfare'. Not necessarily - this is inference. Also poor grammar.
Answer: Thank you for this comment. The sentence has been revised and corrected (Lines 322 and 325).

Comment: L 354-358: You need to exclude coccidia from 'helminthophagous'
Answer: Thank you for noting this. The paragraph has been corrected (Lines 343 to 348).

Reviewer #2:
Comment: The authors have addressed the concerns raised by the referees, and it provides a very useful digest of the state of play regarding the potential role of nematophagous fungi for parasite management. Publication is recommended.
Answer: Thank you very much.

We thank you for the reviewing and guidance through all this process.

Best regards,

Lisbon, 21st June 2020

Miguel Canhão-Dias
Adolfo Paz-Silva
Luís Madeira de Carvalho

***Highlights (for review)**

HIGHLIGHTS

- Systematic review delivering robust evidence on Biological Control of parasites
- New insights on the efficacy of predatory fungi
- New fungi products as promising tools for gastrointestinal parasite control

The efficacy of predatory fungi on the control of gastrointestinal parasites in domestic and wild animals – a systematic review

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Abstract

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The aim of this work was to assess the evidence of efficacy of nematophagous fungi in the control of nematodes and other gastrointestinal parasites in different animal species.

Methods: Using the PICO method (Population, Intervention, Comparison and Outcomes), ~~We~~ we performed a systematic review on the subject to search~~,~~ ~~applying the PICO method (Population, Intervention, Comparison and Outcomes), searching~~ for original papers published between January 2006 and October 2019, written in English, and indexed in PubMed/Medline. Medical Subject Headings (MeSH) terms were used in the syntax. Papers were selected for detailed review based on title and abstract. Inclusion and exclusion criteria were applied, and relevant data ~~was~~ were collected from the remaining papers.

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Conclusion: With the increasing hazards of drug resistance and ecotoxicity, biological control with predatory fungi stands out as a good tool for future parasite management, whether as a complementary treatment or as an alternative to standard parasite control.

Keywords: Animals, Biological control, *Duddingtonia flagrans*, Gastrointestinal parasites, Predatory fungi.

Introduction

Livestock parasites reduce productivity and are a source of economic losses that can reach tens of billions of dollars worldwide (Roeber et al., 2013). Gastrointestinal parasites, namely nematodes, can have a significant impact on animal health and welfare, causing poor growth rates, diseases, and even death (Larsen, 2006).

The traditional approach to the control of this problem has been the use of anthelmintic drugs, which can present some drawbacks, such as ecotoxicity (Vokřál et al., 2019) and the development of multidrug-resistant parasite populations against most anthelmintic classes (Canever et al., 2013; Peregrine et al., 2014). Simultaneously, the increasing demand for food products produced with reduced chemical use and under eco-friendly standards (Clark et al., 2017; Wee et al., 2012) has further demonstrated the need for alternative types of gastrointestinal parasite control.

This situation has stimulated research in strategies based on natural approaches involving pasture management, rotation of animal species, nutritional optimization, administration of copper oxide wire particles or the utilization of natural antagonists. Nematophagous fungi are microorganisms able to reduce the population of parasites, without damaging the host animals (Buzatti et al., 2015; Hernández et al., 2016). *Duddingtonia flagrans* has been considered the most promising fungal species for the control of nematode larvae (Larsen, 2006; Sahoo and Khan, 2016). However, it is not the only species with demonstrated efficacy, and fungi such as *Arthrobotrys robusta* and *Monacrosporium thaumasium* have also been described as useful tools in the control of parasites (Faedo et al., 1997; Braga and Araújo, 2014). These fungi are able to catch the larvae of some nematodes in the soil through trap formation with their mycelia, and then digesting the parasite (Buzatti et al., 2015). Some species of fungi (*Pochonia chlamydosporia*, *Mucor circinelloides*, *Purpureocillium lilacinum*, *Trichoderma* spp.) have shown activity against the eggs of helminths and even coccidian oocysts, due to their ability to attach to their eggshells, penetrate and destroy them (Braga and De Araújo, 2014; J. Á. Hernández et al., 2018a).

Despite the increasing number of studies and publications about the biological control of helminths, there is no systematic review gathering data from the different trials and analyseis on the efficacy of these fungi. In fact, accumulated knowledge arising from research has not yet been translated to daily clinical practice, due to the lack of systematic evidence of efficacy, as well as the scarcity of protocols with standard doses, frequency and duration of treatment, and vehicle of administration.

Systematic reviews provide results from carefully designed intervention studies (controlled trials) and deliver a high level of evidence on the efficacy of specific health interventions. The PICO strategy (Population, Intervention, Comparison and Outcome) pre-defines what are the studies and variables to be included in the analysis, ensuring the high accuracy of the search and quality of the final results (O'Connor et al., 2014; Sargeant and O'Connor, 2014).

The aim of this study was to assess the evidence of efficacy ~~of using~~on the use of nematophagous fungi in the control of nematodes in different animal species and in various contexts, and also to present new insights on their efficacy against other gastrointestinal parasites. The information gathered might be used in the future to support recommendations and guidelines in the area of biological control of parasites. To the authors' best knowledge, this is the first systematic review regarding this subject and covering several species of predatory fungi and hosts.

Material & Methods

The systematic review comprised original papers published between January 2006 and October 2019. Title and abstract selection ~~was~~ were performed in accordance with the PICO strategy and the review question was defined by the PICO components (Table 1).

The literature search phase was conducted through the PubMed/MEDLINE database, combining relevant keywords and MeSH Terms. The full search string carried out was "(biological control OR eggs OR larvae) AND (predatory fungi OR predacious fungi OR nematophagous fungi OR duddingtonia OR arthrobotrys)".

Title and abstract analyses were performed. Articles in languages other than English, reviews, short communications, letters, and editorials were excluded. In addition, papers published before January 2006 were not included.

Eligible studies were submitted to detailed review and PICO criteria ~~was~~ were applied. In the end, relevant data ~~was~~ were collected. We divided and presented the papers by animal species, and for each one, the fungi used, the parasites they were targeting, dose, frequency and treatment duration, measured outcome (e.g., egg reduction, larvae reduction) and results were reported. Other variables analyzed included the type of study (*in vitro*, *in vivo*), country, time of the year, and the vehicle of administration.

Results

The literature search retrieved 616 papers. Eighty-nine were submitted to a detailed review. In the end, 53 papers were included in the analysis, and relevant data ~~was~~ were collected (Figure 1).

In Table 2, the studies' characteristics regarding animal species, fungi used, parasites targeted, dose, frequency, duration and form of administration and evaluated outcomes are detailed. Accordingly, a total of 15 intervention studies were reported on ovine (Aguilar-Marcelino et al., 2016; Aguilar et al., 2008; Da Silva et al., 2015; Eysker et al., 2006a, 2006b; Faessler et al., 2007; Gómez-Rincón et al., 2006; Kahn et al., 2007; Mendoza-De-Gives et al., 2006; Rocha et al., 2007; Sagüés et al., 2011; Silva et al., 2009, 2010, 2011; Waller et al., 2006), 10 on bovines (Assis et al., 2013,

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2012; Bilotto et al., 2018; de Castro Oliveira et al., 2018; Dias et al., 2013; Luns et al., 2018; Mendoza-de Gives et al., 2018; Ortiz Pérez et al., 2017; Silva et al., 2013; Vieira et al., 2019), 8 on equids (Araujo et al., 2010; Braga et al., 2010; Braga et al., 2009a; Buzatti et al., 2015; Hernández et al., 2016, 2018; Tavela et al., 2011, 2013), 7 on canids (Araujo et al., 2013; F. R. Braga et al., 2009b; Carvalho et al., 2009; de Freitas Soares et al., 2015; Maciel et al., 2009, 2010, 2012), 5 on caprine (Cai et al., 2017; Gómez-Rincón et al., 2007; Paraud et al., 2006, 2007; Vilela et al., 2012), 2 on swine (Facchini Rodrigues et al., 2018; Ferreira et al., 2011), 1 on hamster (Fernandes et al., 2012), 1 on feline (Braga et al., 2011), 1 on wild equids (Palomero et al., 2018), 1 hen (Hiura et al., 2015), and 2 papers with intervention in more than one animal species (Arias et al., 2013; Healey et al., 2018). Thirty studies were conducted in Brazil, 7 in Mexico, 5 in Spain, 2 in Australia, 2 in Argentina, 2 in France, 2 in the Netherlands, 1 in China, 1 in Switzerland and 1 in Sweden.

Out of the 53 studies, 23 used the fungi as a single administration, 16 administered it daily, two every 2 days, 11 twice per ~~/~~week, and one every 3 days. Distinct fungi, dosage, frequency and vehicle of administration, treatment duration, outcomes and efficacy were reported. *D. flagrans* (larvicide) was tested in 43 studies (81.1%), six of which in association with other fungi (14%). Associations of fungi were made with *D. flagrans*, *M. circinelloides*, *M. thaumasium*, *A. robusta*, and *Arthrobotrys cladodes*. The frequency and duration of administration protocols varied from a single administration to a 16-month period with daily fungal intake.

The fungal doses used varied between $1.5x10^5$ and $5x10^6$ chlamydospores/ kg of live weight, or between 0.1 and 0.25 grams of mycelia/ 10 kg of live weight, with the

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reductions in Eggs Per Gram and L3 varying between 0 and 100%. Only nine studies (17%) did not show significant differences between the intervention and control groups (six in sheep (Eysker et al., 2006a, 2006b; Faessler et al., 2007; Rocha et al., 2007; Silva et al., 2010; Waller et al., 2006), and the other three in horses, goats, and bovines (Buzatti et al., 2015; Mendoza-de Gives et al., 2018; Paraud et al., 2007)). The explanations for poor results vary, and these could be dependent on low temperatures and initial overall low parasite loads.

~~The vast majority,~~A total of 44 studies (83%)~~,~~ demonstrated efficacy. In the sheep studies, 100% targeted Trichostrongylidae and 13% Chabertidae. In bovines, Trichostrongylidae were targeted in 90% of the studies and Chabertidae in 80%. Strongylidae were targeted in 87,5% of the horse studies, Cyathostominae in 75% and Ascarididae in 12,5%. In the dog studies, the most targeted parasites were Ancylostomatidae (43%), followed by Angiostrongylidae and Ascarididae (29% each). In studies with goats, 100% were targeted to Trichostrongylidae and 20% to Chabertidae. More details can be found in Supplementary Tables 1 – 7.

Discussion

Systematic reviews are the best way to properly collect and gather data, in order to retrieve the strongest evidence from the literature (Sargeant and O'Connor, 2014). The current review confirmed that treatments with nematophagous fungi reduce the number and viability of gastrointestinal parasites' larvae and eggs, both ~~in~~ *in vivo* and *in vitro* experiments, in distinct animal species. The reviewed papers showed very satisfactory results in reducing the need for frequent use of anthelminthic drugs. Authors also reported an increase in time between deworming when using *D. flagrans*

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and *M. circinelloides* in horses (Hernández et al., 2016, 2018) and wild equids (Palomero et al., 2018).

In total, 17% of the studies did not show significant differences between treated and control groups. Explanations for the negative results are debatable. Some authors argued that the dilution of spores due to ~~a~~ greater food consumption by lactating animals might explain the poor parasite reduction (Eysker et al., 2006b). Others defended that the low temperatures during the trials compromised fungal growth (Paraud et al., 2007)~~. Finally~~, while others found no significant efficacy comparing to the control group because the good health of the animals and the parasitological programs already in place prevented the fungal activity to stand out (Waller et al., 2006). However, the range of fungal doses used ~~were~~ was not pointed as a reason~~s~~ for the negative results.

D. flagrans (larvicide) was the most frequently used fungus, alone or in combination with other larvicide or ovicide fungi. Good results were obtained by administering a combination of two or more fungi (Arias et al., 2013; J. Á. Hernández et al., 2018a; Luns et al., 2018; Palomero et al., 2018), even though it is important to note that not all associations were effective. For example, a decreased effectiveness was found when combining *D. flagrans* with *Clonostachys rosea* against *Haemonchus contortus* larvae *in vitro* (Da Silva et al., 2015). Contrarily, Arias et al. (2013) found no antagonism between *M. circinelloides* and *D. flagrans* growing *in vitro* in the same plate. The existing interactions between different nematophagous fungi are not yet completely understood. Nevertheless, in the majority of studies, ~~an~~ increased effectiveness was found when combining different fungi. There is also evidence of

differences in efficacy between isolates of the same fungal species (Cai et al., 2017; Silva et al., 2013). More information is needed to determine the reason why this happens, and which isolates produce the best results. Paz-Silva et al. (2011) have shownned that *D. flagrans* can adapt to higher cyathostomin egg-outputs, maintaining great results in the reduction of third-stage larvae (L3).

Besides the already tested effect of *D. flagrans* on third-stage larvaeL3 of gastrointestinal nematodes, there are reports of its predatory activity against *Angiostrongylus vasorum* first-stage larvae (L1) (Braga et al., 2009b) and *Toxocara canis* second-stage larvae (L2) (Hiura et al., 2015). Using crude extracts from *M. thaumasium,* de Freitas Soares et al. (2015) have shown a reduction of *A. vasorum* first-stage larvae (L1) *in vitro*. This is a new approach to parasite control, namely for pets, that deserves further investigation.

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Fitz-Aranda et al. (2015) stated that some of the biggest factors influencing the success of this kind of treatment are the medium in which the fungi are administered, the ingestion of an effective dose, as well as a storage method that does not alter the fungi's properties. Mixing spores into a nutrient block is an approach already tested, but it has shown the downside of offering a very variable spore intake among animals in the same group, with the ingestion also depending on climate and grazing season (Sagüés et al., 2011). Additionally, the blocks have a relatively high level of moisture comparing to other forms of administration, reducing the product's shelf life (Larsen, 2006). The use of cereal grains combined with chlamydospores has similarly shown to be effective (Facchini Rodrigues et al., 2018; Waller et al., 2001b).

The use of intraruminal Controlled Release Devices has demonstrated good results in ruminants (Sagüés et al., 2014) and presents itself as a good option for future formulations, especially for free-ranging animals (Waller et al., 2001a). Buzatti et al. (2015) showed that administrations with 3-day intervals in horses are effective in reducing the amount of fecal cyathostomin L3, which is also a good prospect for extensive grazers.

Nowadays, most studies incorporate the fungi in edible pellets, namely in a sodium alginate matrix, with good results. The fungi retain their nematophagous activity after passing through the animals' gastrointestinal tract and the pellets allow for an easy dosing and ~~a~~ more even ingestion within the herd; moreover, they are easy to store and preserve (Fitz-Aranda et al., 2015; Hernández et al., 2016). Regarding non-herbivores, an effective reduction of L3 can be achieved by mixing mycelia with regular canned food (Carvalho et al., 2009). However, the use of nematophagous fungi in small domestic animals like dogs and cats in a big scale is still a distant reality, due to the lack of a reliable method of administering a standard dose.

A treatment with nematophagous fungi can be used in combination with other forms of biological control. Hernández et al. (2018) combined *D. flagrans* and *M. circinelloides* chlamydospores with a pasture rotation system, showing a reduction of fecal Eggs Per Gram (EPG) of up to 99% in the first six months of fungal administration in horses. Moreover, they registered an Egg Reappearance Period of 16 weeks after one ivermectin administration associated with the fungi, in comparison with 6 weeks in the control group using only ivermectin. ~~Similarly, the removal of feces from pasture can have good results in worm control (Paz-Silva et al., 2011).~~ It is

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also suggested that animals with a high parasitic burden must be identified and removed from the herd (Rocha et al., 2007). The concomitant administration of chlamydospores and an energetic supplement was successful in reducing the EPG, parasite load, and pasture contamination in goats, compared to a group receiving only chlamydospores (Gómez-Rincón et al., 2007).

It has been suggested that abiotic factors like rain, temperature, and sunlight might influence the performance of the nematophagous fungi. Field trials performed with *D. flagrans* in horses, registered better results in Spring/Summer, than in Autumn/Winter, concerning L3 reduction levels in fecal samples and pasture (Madeira de Carvalho et al., 2007). Indeed, several studies show different fungal efficacies depending on ~~the~~ ambient temperature. Paraud et al. (2006) obtained higher reductions of *H. contortus*, *T. circumcincta* and *T. colubriformis* by *D. flagrans* at 21ºC than at 28ºC. Also, Bilotto et al. (2018) achieved 63.77% and 88.39% L3 reduction in pasture in sunny and shaded conditions in summer, respectively, versus 1,58% and 3.56% in winter. This shows that the relation between temperature, larval development, and fungal efficacy needs to be understood, in order to design successful parasite-management programs.

An interesting question lies in the acquisition of useful strains of fungi with parasiticide activity. Due to only two commercial formulations being available (Healey et al., 2018, Braga et al., 2020), with others in progress, most of the information available has been collected by using fungi isolated in different countries. Soto-Barrientos et al. (2011) successfully used three techniques to isolate nematophagous fungi directly from several farms' soils, an alternative to buying

commercially formulated fungi. More studies are needed to evaluate the advantages and disadvantages of using locally isolated fungi; some important questions would be: if the ecological impacts on the soil are minimized by using fungi already existing in the same environment; if it is cheaper to isolate than to buy; if ~~it~~ there is ~~better to use~~greater efficacy by using ~~a~~ local fungal species/strains, instead of introducing ~~an~~ exotic ones.

It must be noted that regularly used antiparasitic drugs, namely ivermectin and albendazole, do have an *in vitro* inhibitory effect over nematophagous fungi, namely on the development of *Arthrobotrys oligospora, D. flagrans* and *P. lilacinum* (Vieira et al., 2016). Although not likely to occur due to the buffering effect of the rumen, studies should be performed in order to understand if these effects also occur *in vivo*.

From the 53 analyzed articles, only six (11.3%) (Assis et al., 2012, 2013; Dias et al., 2013; Hernández et al., 2016, 2018; Palomero et al., 2018) reported data regarding the administration of fungi for 12 or more months. This is elucidative of the lack of studies that measure the long-term impact of nematophagous fungi on a parasite population. All these studies showed a good long-term reduction of EPG, whether by using ovicidal, larvicidal, or a mixture of both types of fungi. One short-term study (Thapa et al., 2018) has shown an unwanted effect when using *P. chlamydosporia* (ovicidal) against an ascarid parasite of chickens: the reduced parasite burden caused by the exposure to *P. chlamydosporia* led to ~~a~~ faster development of the existing larvae into adult worms within the chicken's ~~organism~~intestine. That, in turn meant a higher EPG and a greater contamination of the environment. This is an extremely important point to have in consideration when designing parasite control programs,

since it may increase the possibility of environment recontamination. More studies need to be conducted in order to fully understand how beneficial these treatments are in the long-term.

From 13 studies that measured ~~animal~~ weight ~~changes~~variations, six (46.2%) showed animals treated with fungi had significantly higher gains ~~in animals treated with fungi~~ when compared to control groups~~, which translates a better overall welfare in these animals~~. The authors have not reported adverse reactions to *D. flagrans* and *M. circinelloides* in horses (Hernández et al., 2016), nor against *P. chlamydosporia* in chickens (Thapa et al., 2018).

New products using nematophagous fungi are being developed. Bioverm® is a powder containing 10^5 *D. flagrans* chlamydospores per gram that has ~~been showing~~shown over 90% efficacy in the reduction of *Strongyloides papillosus* and *H. contortus* larvae in sheep (Braga et al., 2020). Bioworma® is another *D. flagrans* chlamydospore-based product already commercialized and administered at a concentration of $3x10^4$ chlamydospores per kg of body weight. With a much smaller dose than that regularly used, it is capable of reducing the number of viable larvae of several nematodes in horses, goats, and cows (Healey et al., 2018). Further studies are needed to determine the possibility of driving down costs by reducing the number of chlamydospores used~~,~~ while maintaining the same parasite reduction efficacy. In this case, the frequency of dosage should also be taken into account.

Recent studies found that *P. chlamydosporia* can also be useful against eggs of tapeworms (Araujo et al., 2009; Braga et al., 2011) and trematodes (De et al., 2008;

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Dias et al., 2013). These reports support Braga and Araújo (2014) in arguing that nematophagous fungi can start being called helmintophagous. If the results showing *D. flagrans*' efficacy and even against coccidians (Magalhães da Cruz, 2015) are reproduced, they can even be. ~~These reports support Braga and Araújo (2014) in arguing that nematophagous fungi can start being called helmintophagous or,~~ more broadly called predatory fungi.

The fungal doses used varied widely, but no significant correlation between low fungal intake and poor outcomes was found with the doses studied. Although several regimens of fungal administration have been tested and overall good results have been achieved, optimal parasite control is expected to be attained when adopting long term schedules, with at least a twice-per-week fungal administration, as showed by Assis et al. (2013), Hernández et al. (2016) and Silva et al. (2009).

In general, papers with negative or absent results tend to be less published, which can limit the information retrieved by systematic reviews. Despite all the advances in understanding predatory fungi, some questions remain unaddressed. There is scarce information about the fungi's potential impact on the soil microbiome. The fungi's attack mechanisms are based on physical and chemical actions simpler than those of anthelmintic drugs. This could be associated with a decreased rate of development of parasite resistance to predatory fungi. Likewise, there is a need to further evaluate the possibility of predatory fungi infecting immunocompromised humans, limiting their potential use. Consequently, this will continue to be a hot topic within the veterinarian community for the coming years. But it will also be a vanguard tool for animal parasite control with great potential in the future, namely because it is an ecological,

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economical and sustainable approach for doing it in times when ~~less~~ fewer residues in animal tissues and in the environment are a must for animal and human health.

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References

Aguilar-Marcelino, L., Mendoza-De-Gives, P., Torres-Hernández, G., López-Arellano, M.E., Becerril-Pérez, C.M., Orihuela-Trujillo, A., Torres-Acosta, J.F.J., Olmedo-Juárez, A., 2017. Consumption of nutritional pellets with *Duddingtonia flagrans* fungal chlamydospores reduces infective nematode larvae of *Haemonchus contortus* in faeces of Saint Croix lambs. J. Helminthol. 91, 665–671. https://doi.org/10.1017/S0022149X1600081X

Aguilar, J.A.C., De Gives, P.M., López-Arellano, M.E., Liébano Hernández, E., 2008. Evaluation of multinutritional pellets containing *Duddingtonia flagrans*

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chlamydospore for the control of ovine haemonchosis. Ann. N. Y. Acad. Sci. 1149, 161–163. https://doi.org/10.1196/annals.1428.076

Araujo, J.M., Araújo, J.V. de, Braga, F.R., Carvalho, R.O., Ferreira, S.R., 2009. Activity of the nematophagous fungi *Pochonia chlamydosporia*, *Duddingtonia flagrans* and *Monacrosporium thaumasium* on egg capsules of *Dipylidium caninum*. Vet. Parasitol. 166, 86–89. https://doi.org/10.1016/j.vetpar.2009.08.003

Araujo, J.M., Araújo, J.V. de, Braga, F.R., Ferreira, S.R., Tavela, A. de O., 2013. Predatory activity of chlamydospores of the fungus *Pochonia chlamydosporia* on *Toxocara canis* eggs under laboratory conditions. Rev. Bras. Parasitol. Veterinária 22, 171–174. https://doi.org/10.1590/s1984-29612013000100033

Araujo, J.M., Araújo, J. V., Braga, F.R., Carvalho, R.O., 2010. In vitro predatory activity of nematophagous fungi and after passing through gastrointestinal tract of equine on infective larvae of *Strongyloides westeri*. Parasitol. Res. 107, 103–108. https://doi.org/10.1007/s00436-010-1841-y

Arias, M.S., Cazapal-Monteiro, C.F., Suárez, J., Miguélez, S., Francisco, I., Arroyo, F.L., Suárez, J.L., Paz-Silva, A., Sánchez-Andrade, R., Mendoza de Gives, P., 2013. Mixed Production of Filamentous Fungal Spores for Preventing Soil-Transmitted Helminth Zoonoses: A Preliminary Analysis. Biomed Res. Int. 2013, 1–8. https://doi.org/10.1155/2013/567876

Assis, R.C.L., Luns, F.D., Araújo, J. V., Braga, F.R., 2012. Biological control of trichostrongyles in beef cattle by the nematophagous fungus *Duddingtonia flagrans* in tropical southeastern Brazil. Exp. Parasitol. 132, 373–377. https://doi.org/10.1016/j.exppara.2012.08.013

Assis, R.C.L., Luns, F.D., Araújo, J. V., Braga, F.R., Assis, R.L., Marcelino, J.L.,

Formatted: English (United States)

Formatted: English (United States)

Freitas, P.C., Andrade, M.A.S., 2013. Comparison between the action of nematode predatory fungi *Duddingtonia flagrans* and *Monacrosporium thaumasium* in the biological control of bovine gastrointestinal nematodiasis in tropical southeastern Brazil. Vet. Parasitol. 193, 134–140. https://doi.org/10.1016/j.vetpar.2012.12.005

Bilotto, F., Fusé, L.A., Sagües, M.F., Iglesias, L.E., Fernández, A.S., Zegbi, S., Guerrero, I., Saumell, C.A., 2018. Predatory effect of *Duddingtonia flagrans* on infective larvae of gastro-intestinal parasites under sunny and shaded conditions. Exp. Parasitol. 193, 27–32. https://doi.org/10.1016/j.exppara.2018.07.016

Braga, Fabio Ribeiro, Araújo, J.V., Silva, A.R., Araujo, J.M., Carvalho, R.O., Tavela, A.O., Campos, A.K., Carvalho, G.R., 2009. Biological control of horse cyathostomin (Nematoda: Cyathostominae) using the nematophagous fungus *Duddingtonia flagrans* in tropical southeastern Brazil. Vet. Parasitol. 163, 335–340. https://doi.org/10.1016/j.vetpar.2009.05.003

Braga, F.R., Araújo, J. V., Silva, A.R., Carvalho, R.O., Araujo, J.M., Ferreira, S.R., Benjamin, L.A., 2010. Predatory activity of the nematophagous fungus *Duddingtonia flagrans* on horse cyathostomin infective larvae. Trop. Anim. Health Prod. 42, 1161–1165. https://doi.org/10.1007/s11250-010-9542-1

Braga, F. R., Carvalho, R.O., Araújo, J.M., Silva, A.R., Araújo, J. V., Lima, W.S., Tavela, A.O., Ferreira, S.R., 2009. Predatory activity of the fungi *Duddingtonia flagrans*, *Monacrosporium thaumasium*, *Monacrosporium sinense* and *Arthrobotrys robusta* on *Angiostrongylus vasorum* first-stage larvae. J. Helminthol. 83, 303–308. https://doi.org/10.1017/S0022149X09232342

Braga, F.R., De Araújo, J.V., 2014. Nematophagous fungi for biological control of gastrointestinal nematodes in domestic animals. Appl. Microbiol. Biotechnol. 98,

Formatted: English (United States)

71–82. https://doi.org/10.1007/s00253-013-5366-z

Braga, F.R., Ferraz, C.M., Da Silva, E.N., De Araújo, J.V. 2020. Efficiency of the Bioverm® (*Duddingtonia flagrans*) fungal formulation to control in vivo and in vitro of *Haemonchus contortus* and *Strongyloides papillosus* in sheep. 3 Biotech. 10, 62. https://doi.org/10.1007/s13205-019-2042-8

Braga, F.R., Silva, A.R., Carvalho, R.O., Araújo, J. V., Pinto, P.S., 2011. Ovicidal activity of different concentrations of *Pochonia chlamydosporia* chlamydospores on *Taenia taeniaeformis* eggs. J. Helminthol. 85, 7–11. https://doi.org/10.1017/S0022149X10000179

Buzatti, A., de Paula Santos, C., Fernandes, M.A.M., Yoshitani, U.Y., Sprenger, L.K., dos Santos, C.D., Molento, M.B., 2015. *Duddingtonia flagrans* in the control of gastrointestinal nematodes of horses. Exp. Parasitol. 159, 1–4. https://doi.org/10.1016/j.exppara.2015.07.006

Cai, K.Z., Wang, B.B., Xu, Q., Liu, J.L., Wang, K.Y., Xue, Y.J., Zhang, H.Y., Wang, H.Y., Cao, X., Ma, Z.R., 2017. In vitro and in vivo studies of nematophagous fungi *Arthrobotrys musiformis* and *Arthrobotrys robusta* against the larvae of the trichostrongylides. Acta Parasitol. 62, 666–674. https://doi.org/10.1515/ap-2017-0080

Canever, R.J., Braga, P.R.C., Boeckh, A., Grycajuck, M., Bier, D., Molento, M.B., 2013. Lack of *Cyathostomin* sp. reduction after anthelmintic treatment in horses in Brazil. Vet. Parasitol. 194, 35–39. https://doi.org/10.1016/j.vetpar.2012.12.020

Carvalho, R.O., Arajo, J. V., Braga, F.R., Araujo, J.M., Silva, A.R., Tavela, A.O., 2009. Predatory activity of nematophagous fungi on infective larvae of *Ancylostoma* sp.: Evaluation in vitro and after passing through the

Formatted: English (United States)

gastrointestinal tract of dogs. J. Helminthol. 83, 231–236. https://doi.org/10.1017/S0022149X08158965

Clark, B., Stewart, G.B., Panzone, L.A., Kyriazakis, I., Frewer, L.J., 2017. Citizens, consumers and farm animal welfare: A meta-analysis of willingness-to-pay studies. Food Policy 68, 112–127. https://doi.org/10.1016/j.foodpol.2017.01.006

Da Silva, M.E., Braga, F.R., De Gives, P.M., Uriostegui, M.A.M., Reyes, M., Soares, F.E.D.F., De Carvalho, L.M., Rodrigues, F.B., De Araújo, J.V., 2015. Efficacy of *Clonostachys rosea* and *Duddingtonia flagrans* in Reducing the Haemonchus contortus Infective Larvae. Biomed Res. Int. 2015, 10–14. https://doi.org/10.1155/2015/474879

de Castro Oliveira, I., de Carvalho, L.M., Vieira, Í.S., Campos, A.K., Freitas, S.G., de Araujo, J.M., Braga, F.R., de Araújo, J.V., 2018. Using the fungus *Arthrobotrys cladodes var. macroides* as a sustainable strategy to reduce numbers of infective larvae of bovine gastrointestinal parasitic nematodes. J. Invertebr. Pathol. 158, 46–51. https://doi.org/10.1016/j.jip.2018.09.004

de Freitas Soares, F.E., Ribeiro Braga, F., de Araújo, J.V., dos Santos Lima, W., de Queiroz, J.H., 2015. The nematophagous fungus *Monacrosporium thaumasium* and its nematicidal activity on *Angiostrongylus vasorum*. Rev. Iberoam. Micol. 32, 51–53. https://doi.org/10.1016/j.riam.2013.09.005

De, S., Sanyal, P.K., Sarkar, A.K., Patel, N.K., Pal, S., Mandal, S.C., 2008. Screening for Indian isolates of egg-parasitic fungi for use in biological control of fascioliasis and amphistomiasis in ruminant livestock. J. Helminthol. 82, 271–277. https://doi.org/10.1017/s0022149x08982602

Dias, A.S., Araújo, J. V., Braga, F.R., Puppin, A.C., Perboni, W.R., 2013. *Pochonia chlamydosporia* in the biological control of *Fasciola hepatica* in cattle in

Formatted: English (United States)

Southeastern Brazil. Parasitol. Res. 112, 2131–2136. https://doi.org/10.1007/s00436-013-3372-9

Eysker, M., Bakker, N., Kooyman, F.N.J., Olthuis, S.O., Ploeger, H.W., 2006a. Effect of biological control through the daily application of spores of *Duddingtonia flagrans* in lambs kept under an evasive grazing system in the Netherlands. Vet. Parasitol. 140, 312–320. https://doi.org/10.1016/j.vetpar.2006.04.013

Eysker, M., Bakker, N., van der Hall, Y.A., van Hecke, I., Kooyman, F.N.J., van der Linden, D., Schrama, C., Ploeger, H.W., 2006b. The impact of daily *Duddingtonia flagrans* application to lactating ewes on gastrointestinal nematodes infections in their lambs in the Netherlands. Vet. Parasitol. 141, 91–100. https://doi.org/10.1016/j.vetpar.2006.05.005

Facchini Rodrigues, J.V., Braga, F.R., Campos, A.K., de Carvalho, L.M., Araujo, J.M., Aguiar, A.R., Ferraz, C.M., da Silveira, W.F., Valadão, M.C., de Oliveira, T., de Freitas, S.G., de Araújo, J.V., 2018. *Duddingtonia flagrans* formulated in rice bran in the control of *Oesophagostomum* spp. intestinal parasite of swine. Exp. Parasitol. 184, 11–15. https://doi.org/10.1016/j.exppara.2017.11.001

Faedo, M., Larsen, M., Waller, P.J., 1997. The potential of nematophagous fungi to control the free-living stages of nematode parasites of sheep: Comparison between Australian isolates of *Arthrobotrys* spp. and *Duddingtonia flagrans*. Vet. Parasitol. 72, 149–155. https://doi.org/10.1016/S0304-4017(97)00055-1

Faessler, H., Torgerson, P.R., Hertzberg, H., 2007. Failure of *Duddingtonia flagrans* to reduce gastrointestinal nematode infections in dairy ewes. Vet. Parasitol. 147, 96–102. https://doi.org/10.1016/j.vetpar.2007.03.025

Fernandes, F.M., Araújo, J.V., Braga, F.R., Gazzinelli-Guimarães, P.H., Araujo, J.M., Ferreira, S.R., Carvalho, R.O., Mello, I.N.K. de, Fujiwara, R.T., 2012. In vitro

Formatted: Spanish (Spain, International Sort)

biological control of infective larvae of *Ancylostoma ceylanicum*. Rev. Bras. Parasitol. Veterinária 21, 283–286. https://doi.org/10.1590/s1984-29612012000300018

Ferreira, S.R., de Araújo, J.V., Braga, F.R., Araujo, J.M., Fernandes, F.M., 2011. In vitro predatory activity of nematophagous fungi *Duddingtonia flagrans* on infective larvae of *Oesophagostomum* spp. after passing through gastrointestinal tract of pigs. Trop. Anim. Health Prod. 43, 1589–1593. https://doi.org/10.1007/s11250-011-9848-7

Fitz-Aranda, J.A., Mendoza-De-Gives, P., Torres-Acosta, J.F.J., Liébano-Hernández, E., López-Arellano, M.E., Sandoval-Castro, C.A., Quiroz-Romero, H., 2015. *Duddingtonia flagrans* chlamydospores in nutritional pellets: Effect of storage time and conditions on the trapping ability against *Haemonchus contortus* larvae. J. Helminthol. 89, 13–18. https://doi.org/10.1017/S0022149X13000539

Gómez-Rincón, C., Uriarte, J., Valderrábano, J., 2007. Effect of nematophagous fungus *Duddingtonia flagrans* and energy supplementation on the epidemiology of naturally infected kids. Vet. Res. 38, 141–50.

Gómez-Rincón, C., Uriarte, J., Valderrábano, J., 2006. Efficiency of *Duddingtonia flagrans* against Trichostrongyle infections of sheep on mountain pastures. Vet. Parasitol. 141, 84–90. https://doi.org/10.1016/j.vetpar.2006.05.007

Healey, K., Lawlor, C., Knox, M.R., Chambers, M., Lamb, J., Groves, P., 2018. Field evaluation of *Duddingtonia flagrans* IAH 1297 for the reduction of worm burden in grazing animals: Pasture larval studies in horses, cattle and goats. Vet. Parasitol. 258, 124–132. https://doi.org/10.1016/j.vetpar.2018.06.017

Hendawy, S.H.M., 2018. Immunity to gastrointestinal nematodes in ruminants: effector cell mechanisms and cytokines. J. Parasit. Dis. 42, 471–482.

Formatted: English (United States)

Formatted: Portuguese (Portugal)

Formatted: English (United States)

https://doi.org/10.1007/s12639-018-1023-x

Hernández, J.Á., Arroyo, F.L., Suárez, J., Cazapal-Monteiro, C.F., Romasanta, Á., López-Arellano, M.E., Pedreira, J., de Carvalho, L.M.M., Sánchez-Andrade, R., Arias, M.S., de Gives, P.M., Paz-Silva, A., 2016. Feeding horses with industrially manufactured pellets with fungal spores to promote nematode integrated control. Vet. Parasitol. 229, 37–44. https://doi.org/10.1016/j.vetpar.2016.09.014

Hernández, J. Á., Cazapal-Monteiro, C.F., Arroyo, F.L., Silva, M.I., Palomero, A.M., Paz-Silva, A., Sánchez-Andrade, R., Arias, M.S., 2018a. Biological control of soil transmitted helminths (STHs) in a zoological park by using saprophytic fungi. Biol. Control 122, 24–30. https://doi.org/10.1016/j.biocontrol.2018.04.005

Hernández, J. Á., Sánchez-Andrade, R., Cazapal-Monteiro, C.F., Arroyo, F.L., Sanchís, J.M., Paz-Silva, A., Arias, M.S., 2018b. A combined effort to avoid strongyle infection in horses in an oceanic climate region: Rotational grazing and parasiticidal fungi. Parasites and Vectors 11, 1–8. https://doi.org/10.1186/s13071-018-2827-3

Hiura, E., del Carmen Garcia Lopes, A., da Paz, J.S., Gava, M.G., Flecher, M.C., Colares, M., de Freitas Soares, F.E., da Fonseca, L.A., Lacerda, T., de Araújo, J.V., Braga, F.R., 2015. Fungi predatory activity on embryonated *Toxocara canis* eggs inoculated in domestic chickens (*Gallus gallus domesticus*) and destruction of second stage larvae. Parasitol. Res. 114, 3301–3308. https://doi.org/10.1007/s00436-015-4553-5

Kahn, L.P., Norman, T.M., Walkden-Brown, S.W., Crampton, A., O'Connor, L.J., 2007. Trapping efficacy of *Duddingtonia flagrans* against *Haemonchus contortus* at temperatures existing at lambing in Australia. Vet. Parasitol. 146,

576 83–89. https://doi.org/10.1016/j.vetpar.2007.02.004
577 Larsen, M., 2006. Biological control of nematode parasites in sheep. J. Anim. Sci. 84,
578 133–9.
579 Luns, F.D., Carolina, R., Assis, L., Pinheiro, L., Silva, C., Ferraz, C.M., Braga, F.R.,
580 Araújo, J.V. De, 2018. Coadministration of Nematophagous Fungi for Biological
581 Control over Nematodes in Bovine in the South-Eastern Brazil. Biomed Res. Int.
582 2018, 1–6.
583 Maciel, A.S., Araújo, J. V., Campos, A.K., Lopes, E.A., Freitas, L.G., 2009. Predation
584 of *Ancylostoma* spp. dog infective larvae by nematophagous fungi in different
585 conidial concentrations. Vet. Parasitol. 161, 239–247.
586 https://doi.org/10.1016/j.vetpar.2009.01.015
587 Maciel, A.S., Freitas, L.G., Campos, A.K., Lopes, E.A., Araújo, J. V., 2010. The
588 biological control of *Ancylostoma* spp. dog infective larvae by *Duddingtonia*
589 *flagrans* in a soil microcosm. Vet. Parasitol. 173, 262–270.
590 https://doi.org/10.1016/j.vetpar.2010.06.027
591 Maciel, A.S., Freitas, L.G., Figueiredo, L.D., Campos, A.K., Mello, I.N.K., 2012.
592 Antagonistic activity of the fungus *Pochonia chlamydosporia* on mature and
593 immature *Toxocara canis* eggs. Parasitology 139, 1074–1085.
594 https://doi.org/10.1017/S0031182012000418
595 Madeira de Carvalho, L.M., Gillespie, A.T., Serra, P.M., Bernardo, F.A., Farrim,
596 A.P., 2007. Efficacy of the nematophagous fungi *Duddingtonia flagrans* on the
597 biological control of equine in Ribatejo. Rev. Port. Ciências Veterinárias 102,
598 233–247.
599 Magalhães da Cruz, R., 2015. Activity of fungi on eggs, larvae and oocysts of
600 parasites of wild ungulates and dogs. pp. 68-74. Master's Dissertation, Faculty of

Veterinary Medicine, University of Lisbon, Portugal

Mendoza-De-Gives, P., Zapata-Nieto, C., Liébano Hernández, E., López Arellano, M.E., Herrera-Rodríguez, D., González Garduño, R., 2006. Biological control of gastrointestinal parasitic nematodes using *Duddingtonia flagrans* in sheep under natural conditions in Mexico. Ann. N. Y. Acad. Sci. 1081, 355–359. https://doi.org/10.1196/annals.1373.050

Mendoza-de Gives, P., López-Arellano, M.E., Aguilar-Marcelino, L., Olazarán-Jenkins, S., Reyes-Guerrero, D., Ramírez-Várgas, G., Vega-Murillo, V.E., 2018. The nematophagous fungus *Duddingtonia flagrans* reduces the gastrointestinal parasitic nematode larvae population in faeces of orally treated calves maintained under tropical conditions—Dose/response assessment. Vet. Parasitol. 263, 66–72. https://doi.org/10.1016/j.vetpar.2018.10.001

O'Connor, A.M., Anderson, K.M., Goodell, C.K., Sargeant, J.M., 2014. Conducting Systematic Reviews of Intervention Questions I: Writing the Review Protocol, Formulating the Question and Searching the Literature. Zoonoses Public Health 61, 28–38. https://doi.org/10.1111/zph.12125

Ortiz Pérez, D.O., Sánchez Muñoz, B., Nahed Toral, J., Orantes Zebadúa, M.Á., Cruz López, J.L., Reyes García, M.E., Mendoza de Gives, P., 2017. Using *Duddingtonia flagrans* in calves under an organic milk farm production system in the Mexican tropics. Exp. Parasitol. 175, 74–78. https://doi.org/10.1016/j.exppara.2017.02.009

Palomero, A.M., Hernández, J.A., Cazapal-Monteiro, C.F., Balán, F.A., Silva, M.I., Paz-Silva, A., Sánchez-Andrade, R., Vázquez, M.S.A., 2018. Implementation of Biological Control to the Integrated Control of Strongyle Infection among Wild Captive Equids in a Zoological Park. Biomed Res. Int. 2018, 1–7.

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https://doi.org/10.1155/2018/4267683

Paraud, C., Pors, I., Chartier, C., 2007. Efficiency of feeding *Duddingtonia flagrans* chlamydospores to control nematode parasites of first-season grazing goats in France. Vet. Res. Commun. 31, 305–315. https://doi.org/10.1007/s11259-006-3467-1

Paraud, C., Pors, I., Chicard, C., Chartier, C., 2006. Comparative efficacy of the nematode-trapping fungus *Duddingtonia flagrans* against *Haemonchus contortus*, *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* in goat faeces: Influence of the duration and of the temperature of coproculture. Parasitol. Res. 98, 207–213. https://doi.org/10.1007/s00436-005-0028-4

Paz-Silva, A., Francisco, I., Valero-Coss, R.O., Cortiñas, F.J., Sánchez, J.A., Francisco, R., Arias, M., Suárez, J.L., López-Arellano, M.E., Sánchez-Andrade, R., de Gives, P.M., 2011. Ability of the fungus *Duddingtonia flagrans* to adapt to the cyathostomin egg-output by spreading chlamydospores. Vet. Parasitol. 179, 277–282. https://doi.org/10.1016/j.vetpar.2011.02.014

Peregrine, A.S., Molento, M.B., Kaplan, R.M., Nielsen, M.K., 2014. Anthelmintic resistance in important parasites of horses: Does it really matter? Vet. Parasitol. 201, 1–8. https://doi.org/10.1016/j.vetpar.2014.01.004

Rocha, R.A., Araújo, J.V., Amarante, A.F.T., 2007. Efficacy of the nematode-trapping fungus *Duddingtonia flagrans* against infections by *Haemonchus* and *Trichostrongylus* species in lambs at pasture . J. Helminthol. 81, 387–392. https://doi.org/10.1017/s0022149x07853697

Roeber, F., Jex, A.R., Gasser, R.B., 2013. Impact of gastrointestinal parasitic nematodes of sheep, and the role of advanced molecular tools for exploring epidemiology and drug resistance - An Australian perspective. Parasites and

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651 Vectors 6, 1–13. https://doi.org/10.1186/1756-3305-6-153
652 Sagüés, M.F., Fusé, L.A., Fernández, A.S., Iglesias, L.E., Moreno, F.C., Saumell,
653 C.A., 2011. Efficacy of an energy block containing *Duddingtonia flagrans* in the
654 control of gastrointestinal nematodes of sheep. Parasitol. Res. 109, 707–713.
655 https://doi.org/10.1007/s00436-011-2302-y
656 Sagüés, M.F., Purslow, P., Fernández, A.S., Iglesias, L.E., Fusé, L.A., Saumell, C.A.,
657 2014. The use of soy protein polymers as a release device for nematophagous
658 fungi in the control of parasitic nematodes in ruminants. J. Helminthol. 88, 511–
659 514. https://doi.org/10.1017/S0022149X13000394
660 Sahoo, A., Khan, F., 2016. Nutritional and Biological Control Synergism against
661 Gastrointestinal Nematodes in Small Ruminants. J. Vet. Sci. Anim. Husb. 4, 1–
662 13. https://doi.org/10.15744/2348-9790.4.104
663 Sargeant, J.M., O'Connor, A.M., 2014. Introduction to Systematic Reviews in Animal
664 Agriculture and Veterinary Medicine. Zoonoses Public Health 61, 3–9.
665 https://doi.org/10.1111/zph.12128
666 Silva, A.R., Araújo, J. V., Braga, F.R., Alves, C.D.F., Frassy, L.N., 2011. Activity in
667 vitro of fungal *conidia* of *Duddingtonia flagrans* and *Monacrosporium*
668 *thaumasium* on *Haemonchus contortus* infective larvae. J. Helminthol. 85, 138–
669 141. https://doi.org/10.1017/S0022149X10000362
670 Silva, A.R., Araújo, J. V., Braga, F.R., Frassy, L.N., Tavela, A.O., Carvalho, R.O.,
671 Castejon, F. V., 2009. Biological control of sheep gastrointestinal nematodiasis
672 in a tropical region of the southeast of Brazil with the nematode predatory fungi
673 *Duddingtonia flagrans* and *Monacrosporium thaumasium*. Parasitol. Res. 105,
674 1707–1713. https://doi.org/10.1007/s00436-009-1613-8
675 Silva, B.F., Carrijo-Mauad, J.R., Braga, F.R., Campos, A.K., Araújo, J. V., Amarante,

A.F.T., 2010. Efficacy of *Duddingtonia flagrans* and *Arthrobotrys robusta* in controlling sheep parasitic gastroenteritis. Parasitol. Res. 106, 1343–1350. https://doi.org/10.1007/s00436-010-1805-2

Silva, M.E. da, Araújo, J.V. de, Braga, F.R., Freitas Soares, F.E. de, Rodrigues, D.S., 2013. Control of infective larvae of gastrointestinal nematodes in heifers using different isolates of nematophagous fungi. Rev. Bras. Parasitol. Veterinária 22, 78–83. https://doi.org/10.1590/s1984-29612013005000012

Soto-Barrientos, N., de Oliveira, J., Vega-Obando, R., Montero-Caballero, D., Vargas, B., Hernández-Gamboa, J., Orozco-Solano, C., 2011. In-vitro predatory activity of nematophagous fungi from Costa Rica with potential use for controlling sheep and goat parasitic nematodes. Rev. Biol. Trop. 59, 37–52.

Tavela, A. de O., Araújo, J.V., Braga, F.R., Silva, A.R., Carvalho, R.O., Araujo, J.M., Ferreira, S.R., Carvalho, G.R., 2011. Biological control of cyathostomin (Nematoda: Cyathostominae) with nematophagous fungus *Monacrosporium thaumasium* in tropical southeastern Brazil. Vet. Parasitol. 175, 92–96. https://doi.org/10.1016/j.vetpar.2010.09.035

Tavela, A. de O., de Araújo, J.V., Braga, F.R., da Silveira, W.F., Dornelas e Silva, V.H., Carretta Júnior, M., Borges, L.A., Araujo, J.M., Benjamin, L. dos A., Carvalho, G.R., de Paula, A.T., 2013. Coadministration of sodium alginate pellets containing the fungi *Duddingtonia flagrans* and *Monacrosporium thaumasium* on cyathostomin infective larvae after passing through the gastrointestinal tract of horses. Res. Vet. Sci. 94, 568–572. https://doi.org/10.1016/j.rvsc.2012.11.011

Thapa, S., Thamsborg, S.M., Wang, R., Meyling, N. V., Dalgaard, T.S., Petersen, H.H., Mejer, H., 2018. Effect of the nematophagous fungus *Pochonia*

chlamydosporia on soil content of ascarid eggs and infection levels in exposed hens. Parasites and Vectors 11, 1–11. https://doi.org/10.1186/s13071-018-2898-1

Vieira, Í.S., Oliveira, I.D.C., Campos, A.K., Araújo, J.V. De, 2019. Association and predatory capacity of fungi *Pochonia chlamydosporia* and *Arthrobotrys cladodes* in the biological control of parasitic helminths of bovines. Parasitology 146, 1347–1351. https://doi.org/10.1017/S003118201900060X

Vieira, J.N., Maia Filho, F.S., Ferreira, G.F., Mendes, J.F., Gonçalves, C.L., Villela, M.M., Pereira, D.I.B., Nascente, P.S., 2016. In vitro susceptibility of nematophagous fungi to antiparasitic drugs: interactions and implications for biological control. Brazilian J. Biol. 77, 476–479. https://doi.org/10.1590/1519-6984.15715

Vilela, V.L.R., Feitosa, T.F., Braga, F.R., Araújo, J.V. de, Souto, D.V. de O., Santos, H.E. da S., Silva, G.L.L. da, Athayde, A.C.R., 2012. Biological control of goat gastrointestinal helminthiasis by *Duddingtonia flagrans* in a semi-arid region of the northeastern Brazil. Vet. Parasitol. 188, 127–133. https://doi.org/10.1016/j.vetpar.2012.02.018

Vokřál, I., Michaela, Š., Radka, P., Jiří, L., Lukáš, P., Dominika, S., Kateřina, L., Barbora, S., Lenka, S., 2019. Ivermectin environmental impact: Excretion profile in sheep and phytotoxic effect in *Sinapis alba*. Ecotoxicol. Environ. Saf. 169, 944–949. https://doi.org/10.1016/j.ecoenv.2018.11.097

Waller, P.J., Faedo, M., Ellis, K., 2001a. The potential of nematophagous fungi to control the free-living stages of nematode parasites of sheep: towards the development of a fungal controlled release device. Vet. Parasitol. 102, 299–308.

Waller, P.J., Knox, M.R., Faedo, M., 2001b. The potential of nematophagous fungi to control the free-living stages of nematode parasites of sheep: feeding and block

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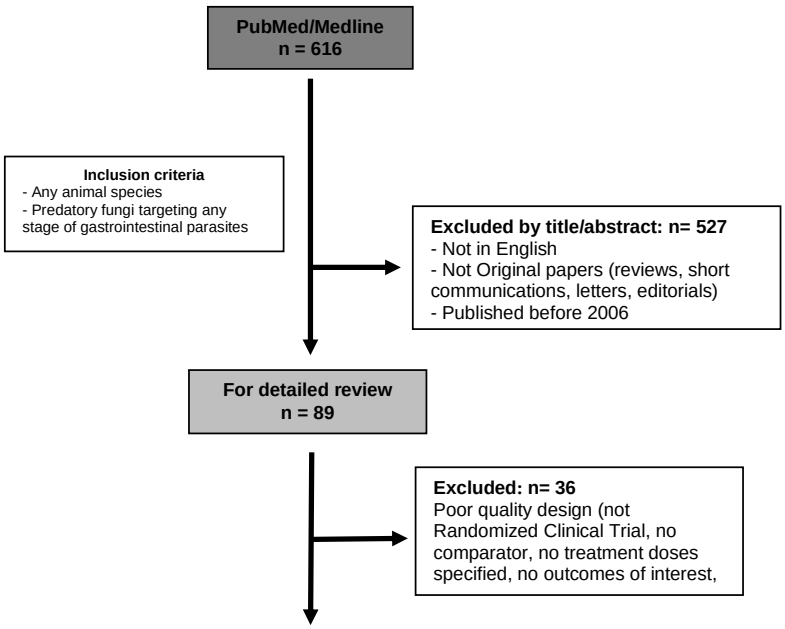
studies with *Duddingtonia flagrans*. Vet. Parasitol. 102, 321–330.

Waller, P.J., Ljungström, B.-L., Schwan, O., Martin, L.R., Morrison, D.A., Rydzik, A., 2006. Biological control of sheep parasites using *Duddingtonia flagrans*: Trials on commercial farms in Sweden. Acta Vet. Scand. 47, 23–32.

Wee, C.S., Ariff, M.S. Bin, Zakuan, N., Tajudin, M.N., 2012. Key Factors Affecting Consumer Purchase Intention a Study of Safe Vegetable in Ho Chi Minh City , Vietnam. Rev. Integr. Bus. Econ. Res. 3, 378–397.



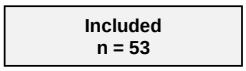


Figure 1- Flowchart with literature search.

Table 1 – PICO method for review question

Population	Gastrointestinal parasites of any animal species.
Intervention	Usage of nematophagous or predatory fungi on any stage of gastrointestinal parasites (e.g., eggs, larvae) in *in vivo*, *in vitro* and *ex vivo* settings. Only fungi that predate gastrointestinal parasites of animals were included.
Comparison	A control group was mandatory. Any relevant comparison was accepted (e.g. placebo, different fungi, different fungus dosage).

Outcome	Quantified control of gastrointestinal parasites (e.g. reduction of number of infective larvae in feces or pasture, reduction of number of eggs in feces or pasture, reduction of parasite load, reduction of deworming frequency). Only papers that quantified the outcomes with figures were included.

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780 Table 2 – Description of studies' characteristics

	Fungi	**Parasites**	**Administration and Dose**	**Frequency and duration**	**Evaluated Outcomes**
Ovine N = 15	*Duddingtonia flagrans* (15); *Monacrosporium thaumasium* (2); *Arthrobotrys robusta* (1); *Clonostachys rosea* (1)	*Haemonchus contortus* (14); *Trichostrongylus* spp. (8); *Teladorsagia* spp. (6) and others	$2x10^5$ – $1x10^6$ chlamydospores /kg live weight (10); Other forms (fixed dose of *mycelia*, *conidia* and chlamydospores)	Single administration (2); Daily for 6 days to 5 months (10); Twice/week for 5 to 6 months (3)	Eggs per gram (EPG) reduction Fecal L3 reduction Reduction of intestinal adult parasites Reduction of L3 /kg pasture dry matter Reduction in Tracer Sheep parasite load
Bovines N = 10	*D. flagrans* (7); *Pochonia*	*Cooperia* spp. (8); *H. contortus* (7);	0,2 – 0,25 g /10 kg live weight (4);	Single administration	EPG reduction Fecal L3 reduction

	chlamydosporia (2); *Arthrobotrys cladodes* (2); *M. thaumasium* (3); *A. robusta* (1)	*Oesophagostomum* spp. (7) and others	Other forms (fixed dose of *mycelia* and chlamydospores)	(4); Daily for 10 days (1); Every 2 days for 30 days (1); Twice/week for 6 to 18 months (4)	Reduction of L3 /kg pasture dry matter
Equids N = 8	*D. flagrans* (7); *M. thaumasium* (3); *A. robusta* (1); *Mucor circinelloides* (2)	*Cyathostominae* (7), *Parascaris* sp. (1); *Strongyloides westeri* (1)	$1,5x10^5 – 2x10^6$ chlamydospores /kg live weight (3); Other forms (fixed dose of *mycelia* and *conidia*)	Single administration (3); Daily for 16 months (1); Twice/week for 21 days to 14 months (4)	EPG reduction Fecal L3 reduction Reduction of L3 /kg pasture dry matter Egg Reappearance Period after Ivermectin administration
Canids N = 7	*D. flagrans* (4); *Arthrobotrys* spp. (6); *Monacrosporium* spp. (6); *P. chlamydosporia* (2)	*Ancylostoma* spp. (3); *Angiostrongylus vasorum*. (2); *Toxocara canis* (2)	Mostly *in vitro*	Single administration (7)	Fecal L1 reduction Fecal L3 reduction Others
Caprine N = 5	*D. flagrans* (4); *Arthrobotrys* spp. (1)	*Trichostrongylus* spp. (5); *Haemonchus* spp. (4); *Teladorsagia* spp. (3) and others	$5x10^5 – 1x10^6$ chlamydospores /kg live weight (4); 0,06g *mycelia* /kg live weight (1)	Single administration (1); Daily for 27 days to 5 months (3); Twice/week for 6 months (1)	EPG reduction Fecal L3 reduction Reduction of intestinal adult parasites Reduction of L3 /kg pasture dry matter Reduction in Tracer Goat parasite load
Swine N = 2	*D. flagrans* (2)	*Oesophagostomum* spp. (2)	Fixed dose of chlamydospores and mycelia (2); $5x10^5$ chlamydospores /kg live weight (1)	Single administration (2)	Fecal L3 reduction
Other N = 6	*D. flagrans* (5); *P. chlamydosporia* (2); *M. circinelloides* (2)	Varies with animal species	Varies with animal species	Single administration (4); Daily for 7 days (1); Every 2 days for 3 years (1)	EPG reduction Fecal L3 reduction Reduction of L3 /kg pasture dry matter Others

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782 N – Number of studies

783 Between brackets are the number of studies with each characteristic

784 EPG – Eggs per gram of feces

785 L1, L3 – parasite larval stages

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The efficacy of predatory fungi on the control of gastrointestinal parasites in domestic and wild animals – a systematic review

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Abstract

Background: Gastrointestinal parasites like nematodes are associated with significant impacts on animal health, causing poor growth rates, diseases and even death. Traditional parasite control includes the use of anthelmintic drugs, albeit being associated with drug resistance and ecotoxicity. In the last decade, biological control of parasites using nematophagous or predatory fungi has been increasingly studied, although systematic evidence of its efficacy is still lacking. The aim of this work was to assess the evidence of efficacy of nematophagous fungi in the control of nematodes and other gastrointestinal parasites in different animal species.

Methods: Using the PICO method (Population, Intervention, Comparison and Outcomes), we performed a systematic review on the subject to search for original papers published between January 2006 and October 2019, written in English, and indexed in PubMed/Medline. Medical Subject Headings (MeSH) terms were used in the syntax. Papers were selected for detailed review based on title and abstract. Inclusion and exclusion criteria were applied, and relevant data were collected from the remaining papers.

Results: The literature search retrieved 616 papers. Eighty-nine were submitted to a detailed review. In the end, 53 papers were included in the analysis. The studies were very heterogeneous, using different fungi, doses, frequency of administration, duration of treatment, host animals, and target parasites. Considering the 53 papers, 44 studies (83% of the interventions) showed efficacy, with only 9 studies (17%) showing no significant differences when compared to control.

Conclusion: With the increasing hazards of drug resistance and ecotoxicity, biological control with predatory fungi stands out as a good tool for future parasite management, whether as a complementary treatment or as an alternative to standard parasite control.

Keywords: Animals, Biological control, *Duddingtonia flagrans*, Gastrointestinal parasites, Predatory fungi.

Introduction

Livestock parasites reduce productivity and are a source of economic losses that can reach tens of billions of dollars worldwide (Roeber et al., 2013). Gastrointestinal parasites, namely nematodes, can have a significant impact on animal health and welfare, causing poor growth rates, diseases, and even death (Larsen, 2006).

The traditional approach to the control of this problem has been the use of anthelmintic drugs, which can present some drawbacks, such as ecotoxicity (Vokřál et al., 2019) and the development of multidrug-resistant parasite populations against most anthelmintic classes (Canever et al., 2013; Peregrine et al., 2014). Simultaneously, the increasing demand for food products produced with reduced chemical use and under eco-friendly standards (Clark et al., 2017; Wee et al., 2012) has further demonstrated the need for alternative types of gastrointestinal parasite control.

This situation has stimulated research in strategies based on natural approaches

involving pasture management, rotation of animal species, nutritional optimization, administration of copper oxide wire particles or the utilization of natural antagonists. Nematophagous fungi are microorganisms able to reduce the population of parasites, without damaging the host animals (Buzatti et al., 2015; Hernández et al., 2016). *Duddingtonia flagrans* has been considered the most promising fungal species for the control of nematode larvae (Larsen, 2006; Sahoo and Khan, 2016). However, it is not the only species with demonstrated efficacy, and fungi such as *Arthrobotrys robusta* and *Monacrosporium thaumasium* have also been described as useful tools in the control of parasites (Faedo et al., 1997; Braga and Araújo, 2014). These fungi are able to catch the larvae of some nematodes in the soil through trap formation with their mycelia, and then digesting the parasite (Buzatti et al., 2015). Some species of fungi (*Pochonia chlamydosporia*, *Mucor circinelloides*, *Purpureocillium lilacinum*, *Trichoderma* spp.) have shown activity against the eggs of helminths and even coccidian oocysts, due to their ability to attach to their eggshells, penetrate and destroy them (Braga and De Araújo, 2014; J. Á. Hernández et al., 2018a).

Despite the increasing number of studies and publications about the biological control of helminths, there is no systematic review gathering data from the different trials and analyses on the efficacy of these fungi. In fact, accumulated knowledge arising from research has not yet been translated to daily clinical practice, due to the lack of systematic evidence of efficacy, as well as the scarcity of protocols with standard doses, frequency and duration of treatment, and vehicle of administration.

Systematic reviews provide results from carefully designed intervention studies (controlled trials) and deliver a high level of evidence on the efficacy of specific

health interventions. The PICO strategy (Population, Intervention, Comparison and Outcome) pre-defines what are the studies and variables to be included in the analysis, ensuring the high accuracy of the search and quality of the final results (O'Connor et al., 2014; Sargeant and O'Connor, 2014).

The aim of this study was to assess the evidence of efficacy on the use of nematophagous fungi in the control of nematodes in different animal species and in various contexts, and also to present new insights on their efficacy against other gastrointestinal parasites. The information gathered might be used in the future to support recommendations and guidelines in the area of biological control of parasites. To the authors' best knowledge, this is the first systematic review regarding this subject and covering several species of predatory fungi and hosts.

Material & Methods

The systematic review comprised original papers published between January 2006 and October 2019. Title and abstract selection were performed in accordance with the PICO strategy and the review question was defined by the PICO components (Table 1).

The literature search phase was conducted through the PubMed/MEDLINE database, combining relevant keywords and MeSH Terms. The full search string carried out was "(biological control OR eggs OR larvae) AND (predatory fungi OR predacious fungi OR nematophagous fungi OR duddingtonia OR arthrobotrys)".

Title and abstract analyses were performed. Articles in languages other than English,

reviews, short communications, letters, and editorials were excluded. In addition, papers published before January 2006 were not included.

Eligible studies were submitted to detailed review and PICO criteria were applied. In the end, relevant data were collected. We divided and presented the papers by animal species, and for each one, the fungi used, the parasites they were targeting, dose, frequency and treatment duration, measured outcome (e.g., egg reduction, larvae reduction) and results were reported. Other variables analyzed included the type of study (*in vitro*, *in vivo*), country, time of the year, and the vehicle of administration.

Results

The literature search retrieved 616 papers. Eighty-nine were submitted to a detailed review. In the end, 53 papers were included in the analysis, and relevant data were collected (Figure 1).

In Table 2, the studies' characteristics regarding animal species, fungi used, parasites targeted, dose, frequency, duration and form of administration and evaluated outcomes are detailed. Accordingly, a total of 15 intervention studies were reported on ovine (Aguilar-Marcelino et al., 2016; Aguilar et al., 2008; Da Silva et al., 2015; Eysker et al., 2006a, 2006b; Faessler et al., 2007; Gómez-Rincón et al., 2006; Kahn et al., 2007; Mendoza-De-Gives et al., 2006; Rocha et al., 2007; Sagüés et al., 2011; Silva et al., 2009, 2010, 2011; Waller et al., 2006), 10 on bovines (Assis et al., 2013, 2012; Bilotto et al., 2018; de Castro Oliveira et al., 2018; Dias et al., 2013; Luns et al., 2018; Mendoza-de Gives et al., 2018; Ortiz Pérez et al., 2017; Silva et al., 2013; Vieira et al., 2019), 8 on equids (Araujo et al., 2010; Braga et al., 2010; Braga et al.,

2009a; Buzatti et al., 2015; Hernández et al., 2016, 2018; Tavela et al., 2011, 2013), 7 on canids (Araujo et al., 2013; F. R. Braga et al., 2009b; Carvalho et al., 2009; de Freitas Soares et al., 2015; Maciel et al., 2009, 2010, 2012), 5 on caprine (Cai et al., 2017; Gómez-Rincón et al., 2007; Paraud et al., 2006, 2007; Vilela et al., 2012), 2 on swine (Facchini Rodrigues et al., 2018; Ferreira et al., 2011), 1 on hamster (Fernandes et al., 2012), 1 on feline (Braga et al., 2011), 1 on wild equids (Palomero et al., 2018), 1 hen (Hiura et al., 2015), and 2 papers with intervention in more than one animal species (Arias et al., 2013; Healey et al., 2018). Thirty studies were conducted in Brazil, 7 in Mexico, 5 in Spain, 2 in Australia, 2 in Argentina, 2 in France, 2 in the Netherlands, 1 in China, 1 in Switzerland and 1 in Sweden.

Out of the 53 studies, 23 used the fungi as a single administration, 16 administered it daily, two every 2 days, 11 twice per week, and one every 3 days. Distinct fungi, dosage, frequency and vehicle of administration, treatment duration, outcomes and efficacy were reported. *D. flagrans* (larvicide) was tested in 43 studies (81.1%), six of which in association with other fungi (14%). Associations of fungi were made with *D. flagrans*, *M. circinelloides*, *M. thaumasium*, *A. robusta*, and *Arthrobotrys cladodes*. The frequency and duration of administration protocols varied from a single administration to a 16-month period with daily fungal intake.

The fungal doses used varied between $1.5x10^5$ and $5x10^6$ chlamydospores/ kg of live weight, or between 0.1 and 0.25 grams of mycelia/ 10 kg of live weight, with the reductions in Eggs Per Gram and L3 varying between 0 and 100%. Only nine studies (17%) did not show significant differences between the intervention and control groups (six in sheep (Eysker et al., 2006a, 2006b; Faessler et al., 2007; Rocha et al.,

2007; Silva et al., 2010; Waller et al., 2006), and the other three in horses, goats, and bovines (Buzatti et al., 2015; Mendoza-de Gives et al., 2018; Paraud et al., 2007)). The explanations for poor results vary, and these could be dependent on low temperatures and initial overall low parasite loads.

A total of 44 studies (83%) demonstrated efficacy. In the sheep studies, 100% targeted Trichostrongylidae and 13% Chabertidae. In bovines, Trichostrongylidae were targeted in 90% of the studies and Chabertidae in 80%. Strongylidae were targeted in 87,5% of the horse studies, Cyathostominae in 75% and Ascarididae in 12,5%. In the dog studies, the most targeted parasites were Ancylostomatidae (43%), followed by Angiostrongylidae and Ascarididae (29% each). In studies with goats, 100% were targeted to Trichostrongylidae and 20% to Chabertidae. More details can be found in Supplementary Tables 1 – 7.

Discussion

Systematic reviews are the best way to properly collect and gather data, in order to retrieve the strongest evidence from the literature (Sargeant and O'Connor, 2014). The current review confirmed that treatments with nematophagous fungi reduce the number and viability of gastrointestinal parasites' larvae and eggs, both *in vivo* and *in vitro* experiments, in distinct animal species. The reviewed papers showed very satisfactory results in reducing the need for frequent use of anthelminthic drugs. Authors also reported an increase in time between deworming when using *D. flagrans* and *M. circinelloides* in horses (Hernández et al., 2016, 2018) and wild equids (Palomero et al., 2018).

In total, 17% of the studies did not show significant differences between treated and control groups. Explanations for the negative results are debatable. Some authors argued that the dilution of spores due to greater food consumption by lactating animals might explain the poor parasite reduction (Eysker et al., 2006b). Others defended that the low temperatures during the trials compromised fungal growth (Paraud et al., 2007), while others found no significant efficacy comparing to the control group because the good health of the animals and the parasitological programs already in place prevented the fungal activity to stand out (Waller et al., 2006). However, the range of fungal doses used was not pointed as a reason for the negative results.

D. flagrans (larvicide) was the most frequently used fungus, alone or in combination with other larvicide or ovicide fungi. Good results were obtained by administering a combination of two or more fungi (Arias et al., 2013; J. Á. Hernández et al., 2018a; Luns et al., 2018; Palomero et al., 2018), even though it is important to note that not all associations were effective. For example, a decreased effectiveness was found when combining *D. flagrans* with *Clonostachys rosea* against *Haemonchus contortus* larvae *in vitro* (Da Silva et al., 2015). Contrarily, Arias et al. (2013) found no antagonism between *M. circinelloides* and *D. flagrans* growing *in vitro* in the same plate. The existing interactions between different nematophagous fungi are not yet completely understood. Nevertheless, in the majority of studies, increased effectiveness was found when combining different fungi. There is also evidence of differences in efficacy between isolates of the same fungal species (Cai et al., 2017; Silva et al., 2013). More information is needed to determine the reason why this happens, and which isolates produce the best results. Paz-Silva et al. (2011) have

shown that *D. flagrans* can adapt to higher cyathostomin egg-outputs, maintaining great results in the reduction of third-stage larvae (L3).

Besides the already tested effect of *D. flagrans* on L3 of gastrointestinal nematodes, there are reports of its predatory activity against *Angiostrongylus vasorum* first-stage larvae (L1) (Braga et al., 2009b) and *Toxocara canis* second-stage larvae (L2) (Hiura et al., 2015). Using crude extracts from *M. thaumasium,* de Freitas Soares et al. (2015) have shown a reduction of *A. vasorum* L1 *in vitro*. This is a new approach to parasite control, namely for pets, that deserves further investigation.

Fitz-Aranda et al. (2015) stated that some of the biggest factors influencing the success of this kind of treatment are the medium in which the fungi are administered, the ingestion of an effective dose, as well as a storage method that does not alter the fungi's properties. Mixing spores into a nutrient block is an approach already tested, but it has shown the downside of offering a very variable spore intake among animals in the same group, with the ingestion also depending on climate and grazing season (Sagüés et al., 2011). Additionally, the blocks have a relatively high level of moisture comparing to other forms of administration, reducing the product's shelf life (Larsen, 2006). The use of cereal grains combined with chlamydospores has similarly shown to be effective (Facchini Rodrigues et al., 2018; Waller et al., 2001b).

The use of intraruminal Controlled Release Devices has demonstrated good results in ruminants (Sagüés et al., 2014) and presents itself as a good option for future formulations, especially for free-ranging animals (Waller et al., 2001a). Buzatti et al. (2015) showed that administrations with 3-day intervals in horses are effective in

reducing the amount of fecal cyathostomin L3, which is also a good prospect for extensive grazers.

Nowadays, most studies incorporate the fungi in edible pellets, namely in a sodium alginate matrix, with good results. The fungi retain their nematophagous activity after passing through the animals' gastrointestinal tract and the pellets allow for an easy dosing and more even ingestion within the herd; moreover, they are easy to store and preserve (Fitz-Aranda et al., 2015; Hernández et al., 2016). Regarding non-herbivores, an effective reduction of L3 can be achieved by mixing mycelia with regular canned food (Carvalho et al., 2009). However, the use of nematophagous fungi in small domestic animals like dogs and cats in a big scale is still a distant reality, due to the lack of a reliable method of administering a standard dose.

A treatment with nematophagous fungi can be used in combination with other forms of biological control. Hernández et al. (2018) combined *D. flagrans* and *M. circinelloides* chlamydospores with a pasture rotation system, showing a reduction of fecal Eggs Per Gram (EPG) of up to 99% in the first six months of fungal administration in horses. Moreover, they registered an Egg Reappearance Period of 16 weeks after one ivermectin administration associated with the fungi, in comparison with 6 weeks in the control group using only ivermectin. It is also suggested that animals with a high parasitic burden must be identified and removed from the herd (Rocha et al., 2007). The concomitant administration of chlamydospores and an energetic supplement was successful in reducing the EPG, parasite load, and pasture contamination in goats, compared to a group receiving only chlamydospores (Gómez-Rincón et al., 2007).

It has been suggested that abiotic factors like rain, temperature, and sunlight might influence the performance of the nematophagous fungi. Field trials performed with *D. flagrans* in horses, registered better results in Spring/Summer, than in Autumn/Winter, concerning L3 reduction levels in fecal samples and pasture (Madeira de Carvalho et al., 2007). Indeed, several studies show different fungal efficacies depending on ambient temperature. Paraud et al. (2006) obtained higher reductions of *H. contortus*, *T. circumcincta* and *T. colubriformis* by *D. flagrans* at 21ºC than at 28ºC. Also, Bilotto et al. (2018) achieved 63.77% and 88.39% L3 reduction in pasture in sunny and shaded conditions in summer, respectively, versus 1,58% and 3.56% in winter. This shows that the relation between temperature, larval development, and fungal efficacy needs to be understood, in order to design successful parasite-management programs.

An interesting question lies in the acquisition of useful strains of fungi with parasiticide activity. Due to only two commercial formulations being available (Healey et al., 2018, Braga et al., 2020), with others in progress, most of the information available has been collected by using fungi isolated in different countries. Soto-Barrientos et al. (2011) successfully used three techniques to isolate nematophagous fungi directly from several farms' soils, an alternative to buying commercially formulated fungi. More studies are needed to evaluate the advantages and disadvantages of using locally isolated fungi; some important questions would be: if the ecological impacts on the soil are minimized by using fungi already existing in the same environment; if it is cheaper to isolate than to buy; if there is greater efficacy by using local fungal species/strains, instead of introducing exotic ones.

It must be noted that regularly used antiparasitic drugs, namely ivermectin and albendazole, do have an *in vitro* inhibitory effect over nematophagous fungi, namely on the development of *Arthrobotrys oligospora, D. flagrans* and *P. lilacinum* (Vieira et al., 2016). Although not likely to occur due to the buffering effect of the rumen, studies should be performed in order to understand if these effects also occur *in vivo*.

From the 53 analyzed articles, only six (11.3%) (Assis et al., 2012, 2013; Dias et al., 2013; Hernández et al., 2016, 2018; Palomero et al., 2018) reported data regarding the administration of fungi for 12 or more months. This is elucidative of the lack of studies that measure the long-term impact of nematophagous fungi on a parasite population. All these studies showed a good long-term reduction of EPG, whether by using ovicidal, larvicidal, or a mixture of both types of fungi. One short-term study (Thapa et al., 2018) has shown an unwanted effect when using *P. chlamydosporia* (ovicidal) against an ascarid parasite of chickens: the reduced parasite burden caused by the exposure to *P. chlamydosporia* led to faster development of the existing larvae into adult worms within the chicken's intestine. That, in turn meant a higher EPG and a greater contamination of the environment. This is an extremely important point to have in consideration when designing parasite control programs, since it may increase the possibility of environment recontamination. More studies need to be conducted in order to fully understand how beneficial these treatments are in the long-term.

From 13 studies that measured weight variations, six (46.2%) showed animals treated with fungi had significantly higher gains when compared to control groups. The authors have not reported adverse reactions to *D. flagrans* and *M. circinelloides* in

horses (Hernández et al., 2016), nor against *P. chlamydosporia* in chickens (Thapa et al., 2018).

New products using nematophagous fungi are being developed. Bioverm® is a powder containing 10^5 *D. flagrans* chlamydospores per gram that has shown over 90% efficacy in the reduction of *Strongyloides papillosus* and *H. contortus* larvae in sheep (Braga et al., 2020). Bioworma® is another *D. flagrans* chlamydospore-based product already commercialized and administered at a concentration of $3x10^4$ chlamydospores per kg of body weight. With a much smaller dose than that regularly used, it is capable of reducing the number of viable larvae of several nematodes in horses, goats, and cows (Healey et al., 2018). Further studies are needed to determine the possibility of driving down costs by reducing the number of chlamydospores used while maintaining the same parasite reduction efficacy. In this case, the frequency of dosage should also be taken into account.

Recent studies found that *P. chlamydosporia* can also be useful against eggs of tapeworms (Araujo et al., 2009; Braga et al., 2011) and trematodes (De et al., 2008; Dias et al., 2013). These reports support Braga and Araújo (2014) in arguing that nematophagous fungi can start being called helmintophagous. If the results showing *D. flagrans*' efficacy against coccidians (Magalhães da Cruz, 2015) are reproduced, they can even be more broadly called predatory fungi.

The fungal doses used varied widely, but no significant correlation between low fungal intake and poor outcomes was found with the doses studied. Although several regimens of fungal administration have been tested and overall good results have been

achieved, optimal parasite control is expected to be attained when adopting long term schedules, with at least a twice-per-week fungal administration, as showed by Assis et al. (2013), Hernández et al. (2016) and Silva et al. (2009).

In general, papers with negative or absent results tend to be less published, which can limit the information retrieved by systematic reviews. Despite all the advances in understanding predatory fungi, some questions remain unaddressed. There is scarce information about the fungi's potential impact on the soil microbiome. The fungi's attack mechanisms are based on physical and chemical actions simpler than those of anthelmintic drugs. This could be associated with a decreased rate of development of parasite resistance to predatory fungi. Likewise, there is a need to further evaluate the possibility of predatory fungi infecting immunocompromised humans, limiting their potential use. Consequently, this will continue to be a hot topic within the veterinarian community for the coming years. But it will also be a vanguard tool for animal parasite control with great potential in the future, namely because it is an ecological, economical and sustainable approach for doing it in times when fewer residues in animal tissues and in the environment are a must for animal and human health.

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References

Aguilar-Marcelino, L., Mendoza-De-Gives, P., Torres-Hernández, G., López-Arellano, M.E., Becerril-Pérez, C.M., Orihuela-Trujillo, A., Torres-Acosta, J.F.J., Olmedo-Juárez, A., 2017. Consumption of nutritional pellets with *Duddingtonia flagrans* fungal chlamydospores reduces infective nematode larvae of *Haemonchus contortus* in faeces of Saint Croix lambs. J. Helminthol. 91, 665–671. https://doi.org/10.1017/S0022149X1600081X

Aguilar, J.A.C., De Gives, P.M., López-Arellano, M.E., Liébano Hernández, E., 2008. Evaluation of multinutritional pellets containing *Duddingtonia flagrans* chlamydospore for the control of ovine haemonchosis. Ann. N. Y. Acad. Sci. 1149, 161–163. https://doi.org/10.1196/annals.1428.076

Araujo, J.M., Araújo, J.V. de, Braga, F.R., Carvalho, R.O., Ferreira, S.R., 2009. Activity of the nematophagous fungi *Pochonia chlamydosporia*, *Duddingtonia flagrans* and *Monacrosporium thaumasium* on egg capsules of *Dipylidium caninum*. Vet. Parasitol. 166, 86–89. https://doi.org/10.1016/j.vetpar.2009.08.003

Araujo, J.M., Araújo, J.V. de, Braga, F.R., Ferreira, S.R., Tavela, A. de O., 2013. Predatory activity of chlamydospores of the fungus *Pochonia chlamydosporia* on *Toxocara canis* eggs under laboratory conditions. Rev. Bras. Parasitol.

Veterinária 22, 171–174. https://doi.org/10.1590/s1984-29612013000100033

Araujo, J.M., Araújo, J. V., Braga, F.R., Carvalho, R.O., 2010. In vitro predatory activity of nematophagous fungi and after passing through gastrointestinal tract of equine on infective larvae of *Strongyloides westeri*. Parasitol. Res. 107, 103–108. https://doi.org/10.1007/s00436-010-1841-y

Arias, M.S., Cazapal-Monteiro, C.F., Suárez, J., Miguélez, S., Francisco, I., Arroyo, F.L., Suárez, J.L., Paz-Silva, A., Sánchez-Andrade, R., Mendoza de Gives, P., 2013. Mixed Production of Filamentous Fungal Spores for Preventing Soil-Transmitted Helminth Zoonoses: A Preliminary Analysis. Biomed Res. Int. 2013, 1–8. https://doi.org/10.1155/2013/567876

Assis, R.C.L., Luns, F.D., Araújo, J. V., Braga, F.R., 2012. Biological control of trichostrongyles in beef cattle by the nematophagous fungus *Duddingtonia flagrans* in tropical southeastern Brazil. Exp. Parasitol. 132, 373–377. https://doi.org/10.1016/j.exppara.2012.08.013

Assis, R.C.L., Luns, F.D., Araújo, J. V., Braga, F.R., Assis, R.L., Marcelino, J.L., Freitas, P.C., Andrade, M.A.S., 2013. Comparison between the action of nematode predatory fungi *Duddingtonia flagrans* and *Monacrosporium thaumasium* in the biological control of bovine gastrointestinal nematodiasis in tropical southeastern Brazil. Vet. Parasitol. 193, 134–140. https://doi.org/10.1016/j.vetpar.2012.12.005

Bilotto, F., Fusé, L.A., Sagües, M.F., Iglesias, L.E., Fernández, A.S., Zegbi, S., Guerrero, I., Saumell, C.A., 2018. Predatory effect of *Duddingtonia flagrans* on infective larvae of gastro-intestinal parasites under sunny and shaded conditions. Exp. Parasitol. 193, 27–32. https://doi.org/10.1016/j.exppara.2018.07.016

Braga, Fabio Ribeiro, Araújo, J.V., Silva, A.R., Araujo, J.M., Carvalho, R.O., Tavela,

425 A.O., Campos, A.K., Carvalho, G.R., 2009. Biological control of horse
426 cyathostomin (Nematoda: Cyathostominae) using the nematophagous fungus
427 *Duddingtonia flagrans* in tropical southeastern Brazil. Vet. Parasitol. 163, 335–
428 340. https://doi.org/10.1016/j.vetpar.2009.05.003
429 Braga, F.R., Araújo, J. V., Silva, A.R., Carvalho, R.O., Araujo, J.M., Ferreira, S.R.,
430 Benjamin, L.A., 2010. Predatory activity of the nematophagous fungus
431 *Duddingtonia flagrans* on horse cyathostomin infective larvae. Trop. Anim.
432 Health Prod. 42, 1161–1165. https://doi.org/10.1007/s11250-010-9542-1
433 Braga, F. R., Carvalho, R.O., Araújo, J.M., Silva, A.R., Araújo, J. V., Lima, W.S.,
434 Tavela, A.O., Ferreira, S.R., 2009. Predatory activity of the fungi *Duddingtonia*
435 *flagrans, Monacrosporium thaumasium, Monacrosporium sinense* and
436 *Arthrobotrys robusta* on *Angiostrongylus vasorum* first-stage larvae. J.
437 Helminthol. 83, 303–308. https://doi.org/10.1017/S0022149X09232342
438 Braga, F.R., De Araújo, J.V., 2014. Nematophagous fungi for biological control of
439 gastrointestinal nematodes in domestic animals. Appl. Microbiol. Biotechnol. 98,
440 71–82. https://doi.org/10.1007/s00253-013-5366-z
441 Braga, F.R., Ferraz, C.M., Da Silva, E.N., De Araújo, J.V. 2020. Efficiency of the
442 Bioverm® (*Duddingtonia flagrans*) fungal formulation to control in vivo and in
443 vitro of *Haemonchus contortus* and *Strongyloides papillosus* in sheep. 3 Biotech.
444 10, 62. https://doi.org/10.1007/s13205-019-2042-8
445 Braga, F.R., Silva, A.R., Carvalho, R.O., Araújo, J. V., Pinto, P.S., 2011. Ovicidal
446 activity of different concentrations of *Pochonia chlamydosporia* chlamydospores
447 on *Taenia taeniaeformis* eggs. J. Helminthol. 85, 7–11.
448 https://doi.org/10.1017/S0022149X10000179
449 Buzatti, A., de Paula Santos, C., Fernandes, M.A.M., Yoshitani, U.Y., Sprenger, L.K.,

dos Santos, C.D., Molento, M.B., 2015. *Duddingtonia flagrans* in the control of gastrointestinal nematodes of horses. Exp. Parasitol. 159, 1–4. https://doi.org/10.1016/j.exppara.2015.07.006

Cai, K.Z., Wang, B.B., Xu, Q., Liu, J.L., Wang, K.Y., Xue, Y.J., Zhang, H.Y., Wang, H.Y., Cao, X., Ma, Z.R., 2017. In vitro and in vivo studies of nematophagous fungi *Arthrobotrys musiformis* and *Arthrobotrys robusta* against the larvae of the trichostrongylides. Acta Parasitol. 62, 666–674. https://doi.org/10.1515/ap-2017-0080

Canever, R.J., Braga, P.R.C., Boeckh, A., Grycajuck, M., Bier, D., Molento, M.B., 2013. Lack of *Cyathostomin* sp. reduction after anthelmintic treatment in horses in Brazil. Vet. Parasitol. 194, 35–39. https://doi.org/10.1016/j.vetpar.2012.12.020

Carvalho, R.O., Arajo, J. V., Braga, F.R., Araujo, J.M., Silva, A.R., Tavela, A.O., 2009. Predatory activity of nematophagous fungi on infective larvae of *Ancylostoma* sp.: Evaluation in vitro and after passing through the gastrointestinal tract of dogs. J. Helminthol. 83, 231–236. https://doi.org/10.1017/S0022149X08158965

Clark, B., Stewart, G.B., Panzone, L.A., Kyriazakis, I., Frewer, L.J., 2017. Citizens, consumers and farm animal welfare: A meta-analysis of willingness-to-pay studies. Food Policy 68, 112–127. https://doi.org/10.1016/j.foodpol.2017.01.006

Da Silva, M.E., Braga, F.R., De Gives, P.M., Uriostegui, M.A.M., Reyes, M., Soares, F.E.D.F., De Carvalho, L.M., Rodrigues, F.B., De Araújo, J.V., 2015. Efficacy of *Clonostachys rosea* and *Duddingtonia flagrans* in Reducing the Haemonchus contortus Infective Larvae. Biomed Res. Int. 2015, 10–14. https://doi.org/10.1155/2015/474879

de Castro Oliveira, I., de Carvalho, L.M., Vieira, Í.S., Campos, A.K., Freitas, S.G., de Araujo, J.M., Braga, F.R., de Araújo, J.V., 2018. Using the fungus *Arthrobotrys cladodes var. macroides* as a sustainable strategy to reduce numbers of infective larvae of bovine gastrointestinal parasitic nematodes. J. Invertebr. Pathol. 158, 46–51. https://doi.org/10.1016/j.jip.2018.09.004

de Freitas Soares, F.E., Ribeiro Braga, F., de Araújo, J.V., dos Santos Lima, W., de Queiroz, J.H., 2015. The nematophagous fungus *Monacrosporium thaumasium* and its nematicidal activity on *Angiostrongylus vasorum*. Rev. Iberoam. Micol. 32, 51–53. https://doi.org/10.1016/j.riam.2013.09.005

De, S., Sanyal, P.K., Sarkar, A.K., Patel, N.K., Pal, S., Mandal, S.C., 2008. Screening for Indian isolates of egg-parasitic fungi for use in biological control of fascioliasis and amphistomiasis in ruminant livestock. J. Helminthol. 82, 271–277. https://doi.org/10.1017/s0022149x08982602

Dias, A.S., Araújo, J. V., Braga, F.R., Puppin, A.C., Perboni, W.R., 2013. *Pochonia chlamydosporia* in the biological control of *Fasciola hepatica* in cattle in Southeastern Brazil. Parasitol. Res. 112, 2131–2136. https://doi.org/10.1007/s00436-013-3372-9

Eysker, M., Bakker, N., Kooyman, F.N.J., Olthuis, S.O., Ploeger, H.W., 2006a. Effect of biological control through the daily application of spores of *Duddingtonia flagrans* in lambs kept under an evasive grazing system in the Netherlands. Vet. Parasitol. 140, 312–320. https://doi.org/10.1016/j.vetpar.2006.04.013

Eysker, M., Bakker, N., van der Hall, Y.A., van Hecke, I., Kooyman, F.N.J., van der Linden, D., Schrama, C., Ploeger, H.W., 2006b. The impact of daily *Duddingtonia flagrans* application to lactating ewes on gastrointestinal nematodes infections in their lambs in the Netherlands. Vet. Parasitol. 141, 91–

100. https://doi.org/10.1016/j.vetpar.2006.05.005

Facchini Rodrigues, J.V., Braga, F.R., Campos, A.K., de Carvalho, L.M., Araujo, J.M., Aguiar, A.R., Ferraz, C.M., da Silveira, W.F., Valadão, M.C., de Oliveira, T., de Freitas, S.G., de Araújo, J.V., 2018. *Duddingtonia flagrans* formulated in rice bran in the control of *Oesophagostomum* spp. intestinal parasite of swine. Exp. Parasitol. 184, 11–15. https://doi.org/10.1016/j.exppara.2017.11.001

Faedo, M., Larsen, M., Waller, P.J., 1997. The potential of nematophagous fungi to control the free-living stages of nematode parasites of sheep: Comparison between Australian isolates of *Arthrobotrys* spp. and *Duddingtonia flagrans*. Vet. Parasitol. 72, 149–155. https://doi.org/10.1016/S0304-4017(97)00055-1

Faessler, H., Torgerson, P.R., Hertzberg, H., 2007. Failure of *Duddingtonia flagrans* to reduce gastrointestinal nematode infections in dairy ewes. Vet. Parasitol. 147, 96–102. https://doi.org/10.1016/j.vetpar.2007.03.025

Fernandes, F.M., Araújo, J.V., Braga, F.R., Gazzinelli-Guimarães, P.H., Araujo, J.M., Ferreira, S.R., Carvalho, R.O., Mello, I.N.K. de, Fujiwara, R.T., 2012. In vitro biological control of infective larvae of *Ancylostoma ceylanicum*. Rev. Bras. Parasitol. Veterinária 21, 283–286. https://doi.org/10.1590/s1984-29612012000300018

Ferreira, S.R., de Araújo, J.V., Braga, F.R., Araujo, J.M., Fernandes, F.M., 2011. In vitro predatory activity of nematophagous fungi *Duddingtonia flagrans* on infective larvae of *Oesophagostomum* spp. after passing through gastrointestinal tract of pigs. Trop. Anim. Health Prod. 43, 1589–1593. https://doi.org/10.1007/s11250-011-9848-7

Fitz-Aranda, J.A., Mendoza-De-Gives, P., Torres-Acosta, J.F.J., Liébano-Hernández, E., López-Arellano, M.E., Sandoval-Castro, C.A., Quiroz-Romero, H., 2015.

525 *Duddingtonia flagrans* chlamydospores in nutritional pellets: Effect of storage
526 time and conditions on the trapping ability against *Haemonchus contortus* larvae.
527 J. Helminthol. 89, 13–18. https://doi.org/10.1017/S0022149X13000539
528 Gómez-Rincón, C., Uriarte, J., Valderrábano, J., 2007. Effect of nematophagous
529 fungus *Duddingtonia flagrans* and energy supplementation on the epidemiology
530 of naturally infected kids. Vet. Res. 38, 141–50.
531 Gómez-Rincón, C., Uriarte, J., Valderrábano, J., 2006. Efficiency of *Duddingtonia*
532 *flagrans* against Trichostrongyle infections of sheep on mountain pastures. Vet.
533 Parasitol. 141, 84–90. https://doi.org/10.1016/j.vetpar.2006.05.007
534 Healey, K., Lawlor, C., Knox, M.R., Chambers, M., Lamb, J., Groves, P., 2018. Field
535 evaluation of *Duddingtonia flagrans* IAH 1297 for the reduction of worm burden
536 in grazing animals: Pasture larval studies in horses, cattle and goats. Vet.
537 Parasitol. 258, 124–132. https://doi.org/10.1016/j.vetpar.2018.06.017
538 Hendawy, S.H.M., 2018. Immunity to gastrointestinal nematodes in ruminants:
539 effector cell mechanisms and cytokines. J. Parasit. Dis. 42, 471–482.
540 https://doi.org/10.1007/s12639-018-1023-x
541 Hernández, J.Á., Arroyo, F.L., Suárez, J., Cazapal-Monteiro, C.F., Romasanta, Á.,
542 López-Arellano, M.E., Pedreira, J., de Carvalho, L.M.M., Sánchez-Andrade, R.,
543 Arias, M.S., de Gives, P.M., Paz-Silva, A., 2016. Feeding horses with
544 industrially manufactured pellets with fungal spores to promote nematode
545 integrated control. Vet. Parasitol. 229, 37–44.
546 https://doi.org/10.1016/j.vetpar.2016.09.014
547 Hernández, J. Á., Cazapal-Monteiro, C.F., Arroyo, F.L., Silva, M.I., Palomero, A.M.,
548 Paz-Silva, A., Sánchez-Andrade, R., Arias, M.S., 2018a. Biological control of
549 soil transmitted helminths (STHs) in a zoological park by using saprophytic

fungi. Biol. Control 122, 24–30. https://doi.org/10.1016/j.biocontrol.2018.04.005

Hernández, J. Á., Sánchez-Andrade, R., Cazapal-Monteiro, C.F., Arroyo, F.L., Sanchís, J.M., Paz-Silva, A., Arias, M.S., 2018b. A combined effort to avoid strongyle infection in horses in an oceanic climate region: Rotational grazing and parasiticidal fungi. Parasites and Vectors 11, 1–8. https://doi.org/10.1186/s13071-018-2827-3

Hiura, E., del Carmen Garcia Lopes, A., da Paz, J.S., Gava, M.G., Flecher, M.C., Colares, M., de Freitas Soares, F.E., da Fonseca, L.A., Lacerda, T., de Araújo, J.V., Braga, F.R., 2015. Fungi predatory activity on embryonated *Toxocara canis* eggs inoculated in domestic chickens (*Gallus gallus domesticus*) and destruction of second stage larvae. Parasitol. Res. 114, 3301–3308. https://doi.org/10.1007/s00436-015-4553-5

Kahn, L.P., Norman, T.M., Walkden-Brown, S.W., Crampton, A., O'Connor, L.J., 2007. Trapping efficacy of *Duddingtonia flagrans* against *Haemonchus contortus* at temperatures existing at lambing in Australia. Vet. Parasitol. 146, 83–89. https://doi.org/10.1016/j.vetpar.2007.02.004

Larsen, M., 2006. Biological control of nematode parasites in sheep. J. Anim. Sci. 84, 133–9.

Luns, F.D., Carolina, R., Assis, L., Pinheiro, L., Silva, C., Ferraz, C.M., Braga, F.R., Araújo, J.V. De, 2018. Coadministration of Nematophagous Fungi for Biological Control over Nematodes in Bovine in the South-Eastern Brazil. Biomed Res. Int. 2018, 1–6.

Maciel, A.S., Araújo, J. V., Campos, A.K., Lopes, E.A., Freitas, L.G., 2009. Predation of *Ancylostoma* spp. dog infective larvae by nematophagous fungi in different conidial concentrations. Vet. Parasitol. 161, 239–247.

https://doi.org/10.1016/j.vetpar.2009.01.015

Maciel, A.S., Freitas, L.G., Campos, A.K., Lopes, E.A., Araújo, J. V., 2010. The biological control of *Ancylostoma* spp. dog infective larvae by *Duddingtonia flagrans* in a soil microcosm. Vet. Parasitol. 173, 262–270. https://doi.org/10.1016/j.vetpar.2010.06.027

Maciel, A.S., Freitas, L.G., Figueiredo, L.D., Campos, A.K., Mello, I.N.K., 2012. Antagonistic activity of the fungus *Pochonia chlamydosporia* on mature and immature *Toxocara canis* eggs. Parasitology 139, 1074–1085. https://doi.org/10.1017/S0031182012000418

Madeira de Carvalho, L.M., Gillespie, A.T., Serra, P.M., Bernardo, F.A., Farrim, A.P., 2007. Efficacy of the nematophagous fungi *Duddingtonia flagrans* on the biological control of equine in Ribatejo. Rev. Port. Ciências Veterinárias 102, 233–247.

Magalhães da Cruz, R., 2015. Activity of fungi on eggs, larvae and oocysts of parasites of wild ungulates and dogs. pp. 68-74. Master's Dissertation, Faculty of Veterinary Medicine, University of Lisbon, Portugal

Mendoza-De-Gives, P., Zapata-Nieto, C., Liébano Hernández, E., López Arellano, M.E., Herrera-Rodríguez, D., González Garduño, R., 2006. Biological control of gastrointestinal parasitic nematodes using *Duddingtonia flagrans* in sheep under natural conditions in Mexico. Ann. N. Y. Acad. Sci. 1081, 355–359. https://doi.org/10.1196/annals.1373.050

Mendoza-de Gives, P., López-Arellano, M.E., Aguilar-Marcelino, L., Olazarán-Jenkins, S., Reyes-Guerrero, D., Ramírez-Várgas, G., Vega-Murillo, V.E., 2018. The nematophagous fungus *Duddingtonia flagrans* reduces the gastrointestinal parasitic nematode larvae population in faeces of orally treated calves

maintained under tropical conditions—Dose/response assessment. Vet. Parasitol. 263, 66–72. https://doi.org/10.1016/j.vetpar.2018.10.001

O'Connor, A.M., Anderson, K.M., Goodell, C.K., Sargeant, J.M., 2014. Conducting Systematic Reviews of Intervention Questions I: Writing the Review Protocol, Formulating the Question and Searching the Literature. Zoonoses Public Health 61, 28–38. https://doi.org/10.1111/zph.12125

Ortiz Pérez, D.O., Sánchez Muñoz, B., Nahed Toral, J., Orantes Zebadúa, M.Á., Cruz López, J.L., Reyes García, M.E., Mendoza de Gives, P., 2017. Using *Duddingtonia flagrans* in calves under an organic milk farm production system in the Mexican tropics. Exp. Parasitol. 175, 74–78. https://doi.org/10.1016/j.exppara.2017.02.009

Palomero, A.M., Hernández, J.A., Cazapal-Monteiro, C.F., Balán, F.A., Silva, M.I., Paz-Silva, A., Sánchez-Andrade, R., Vázquez, M.S.A., 2018. Implementation of Biological Control to the Integrated Control of Strongyle Infection among Wild Captive Equids in a Zoological Park. Biomed Res. Int. 2018, 1–7. https://doi.org/10.1155/2018/4267683

Paraud, C., Pors, I., Chartier, C., 2007. Efficiency of feeding *Duddingtonia flagrans* chlamydospores to control nematode parasites of first-season grazing goats in France. Vet. Res. Commun. 31, 305–315. https://doi.org/10.1007/s11259-006-3467-1

Paraud, C., Pors, I., Chicard, C., Chartier, C., 2006. Comparative efficacy of the nematode-trapping fungus *Duddingtonia flagrans* against *Haemonchus contortus*, *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* in goat faeces: Influence of the duration and of the temperature of coproculture. Parasitol. Res. 98, 207–213. https://doi.org/10.1007/s00436-005-0028-4

625 Paz-Silva, A., Francisco, I., Valero-Coss, R.O., Cortiñas, F.J., Sánchez, J.A.,
626 Francisco, R., Arias, M., Suárez, J.L., López-Arellano, M.E., Sánchez-Andrade,
627 R., de Gives, P.M., 2011. Ability of the fungus *Duddingtonia flagrans* to adapt
628 to the cyathostomin egg-output by spreading chlamydospores. Vet. Parasitol.
629 179, 277–282. https://doi.org/10.1016/j.vetpar.2011.02.014
630 Peregrine, A.S., Molento, M.B., Kaplan, R.M., Nielsen, M.K., 2014. Anthelmintic
631 resistance in important parasites of horses: Does it really matter? Vet. Parasitol.
632 201, 1–8. https://doi.org/10.1016/j.vetpar.2014.01.004
633 Rocha, R.A., Araújo, J.V., Amarante, A.F.T., 2007. Efficacy of the nematode-
634 trapping fungus *Duddingtonia flagrans* against infections by *Haemonchus* and
635 *Trichostrongylus* species in lambs at pasture . J. Helminthol. 81, 387–392.
636 https://doi.org/10.1017/s0022149x07853697
637 Roeber, F., Jex, A.R., Gasser, R.B., 2013. Impact of gastrointestinal parasitic
638 nematodes of sheep, and the role of advanced molecular tools for exploring
639 epidemiology and drug resistance - An Australian perspective. Parasites and
640 Vectors 6, 1–13. https://doi.org/10.1186/1756-3305-6-153
641 Sagüés, M.F., Fusé, L.A., Fernández, A.S., Iglesias, L.E., Moreno, F.C., Saumell,
642 C.A., 2011. Efficacy of an energy block containing *Duddingtonia flagrans* in the
643 control of gastrointestinal nematodes of sheep. Parasitol. Res. 109, 707–713.
644 https://doi.org/10.1007/s00436-011-2302-y
645 Sagüés, M.F., Purslow, P., Fernández, A.S., Iglesias, L.E., Fusé, L.A., Saumell, C.A.,
646 2014. The use of soy protein polymers as a release device for nematophagous
647 fungi in the control of parasitic nematodes in ruminants. J. Helminthol. 88, 511–
648 514. https://doi.org/10.1017/S0022149X13000394
649 Sahoo, A., Khan, F., 2016. Nutritional and Biological Control Synergism against

650 Gastrointestinal Nematodes in Small Ruminants. J. Vet. Sci. Anim. Husb. 4, 1–
651 13. https://doi.org/10.15744/2348-9790.4.104
652 Sargeant, J.M., O'Connor, A.M., 2014. Introduction to Systematic Reviews in Animal
653 Agriculture and Veterinary Medicine. Zoonoses Public Health 61, 3–9.
654 https://doi.org/10.1111/zph.12128
655 Silva, A.R., Araújo, J. V., Braga, F.R., Alves, C.D.F., Frassy, L.N., 2011. Activity in
656 vitro of fungal *conidia* of *Duddingtonia flagrans* and *Monacrosporium*
657 *thaumasium* on *Haemonchus contortus* infective larvae. J. Helminthol. 85, 138–
658 141. https://doi.org/10.1017/S0022149X10000362
659 Silva, A.R., Araújo, J. V., Braga, F.R., Frassy, L.N., Tavela, A.O., Carvalho, R.O.,
660 Castejon, F. V., 2009. Biological control of sheep gastrointestinal nematodiasis
661 in a tropical region of the southeast of Brazil with the nematode predatory fungi
662 *Duddingtonia flagrans* and *Monacrosporium thaumasium*. Parasitol. Res. 105,
663 1707–1713. https://doi.org/10.1007/s00436-009-1613-8
664 Silva, B.F., Carrijo-Mauad, J.R., Braga, F.R., Campos, A.K., Araújo, J. V., Amarante,
665 A.F.T., 2010. Efficacy of *Duddingtonia flagrans* and *Arthrobotrys robusta* in
666 controlling sheep parasitic gastroenteritis. Parasitol. Res. 106, 1343–1350.
667 https://doi.org/10.1007/s00436-010-1805-2
668 Silva, M.E. da, Araújo, J.V. de, Braga, F.R., Freitas Soares, F.E. de, Rodrigues, D.S.,
669 2013. Control of infective larvae of gastrointestinal nematodes in heifers using
670 different isolates of nematophagous fungi. Rev. Bras. Parasitol. Veterinária 22,
671 78–83. https://doi.org/10.1590/s1984-29612013005000012
672 Soto-Barrientos, N., de Oliveira, J., Vega-Obando, R., Montero-Caballero, D.,
673 Vargas, B., Hernández-Gamboa, J., Orozco-Solano, C., 2011. In-vitro predatory
674 activity of nematophagous fungi from Costa Rica with potential use for

675 controlling sheep and goat parasitic nematodes. Rev. Biol. Trop. 59, 37–52.
676 Tavela, A. de O., Araújo, J.V., Braga, F.R., Silva, A.R., Carvalho, R.O., Araujo, J.M.,
677 Ferreira, S.R., Carvalho, G.R., 2011. Biological control of cyathostomin
678 (Nematoda: Cyathostominae) with nematophagous fungus *Monacrosporium*
679 *thaumasium* in tropical southeastern Brazil. Vet. Parasitol. 175, 92–96.
680 https://doi.org/10.1016/j.vetpar.2010.09.035
681 Tavela, A. de O., de Araújo, J.V., Braga, F.R., da Silveira, W.F., Dornelas e Silva,
682 V.H., Carretta Júnior, M., Borges, L.A., Araujo, J.M., Benjamin, L. dos A.,
683 Carvalho, G.R., de Paula, A.T., 2013. Coadministration of sodium alginate
684 pellets containing the fungi *Duddingtonia flagrans* and *Monacrosporium*
685 *thaumasium* on cyathostomin infective larvae after passing through the
686 gastrointestinal tract of horses. Res. Vet. Sci. 94, 568–572.
687 https://doi.org/10.1016/j.rvsc.2012.11.011
688 Thapa, S., Thamsborg, S.M., Wang, R., Meyling, N. V., Dalgaard, T.S., Petersen,
689 H.H., Mejer, H., 2018. Effect of the nematophagous fungus *Pochonia*
690 *chlamydosporia* on soil content of ascarid eggs and infection levels in exposed
691 hens. Parasites and Vectors 11, 1–11. https://doi.org/10.1186/s13071-018-2898-1
692 Vieira, Í.S., Oliveira, I.D.C., Campos, A.K., Araújo, J.V. De, 2019. Association and
693 predatory capacity of fungi *Pochonia chlamydosporia* and *Arthrobotrys cladodes*
694 in the biological control of parasitic helminths of bovines. Parasitology 146,
695 1347–1351. https://doi.org/10.1017/S003118201900060X
696 Vieira, J.N., Maia Filho, F.S., Ferreira, G.F., Mendes, J.F., Gonçalves, C.L., Villela,
697 M.M., Pereira, D.I.B., Nascente, P.S., 2016. In vitro susceptibility of
698 nematophagous fungi to antiparasitic drugs: interactions and implications for
699 biological control. Brazilian J. Biol. 77, 476–479. https://doi.org/10.1590/1519-

6984.15715

Vilela, V.L.R., Feitosa, T.F., Braga, F.R., Araújo, J.V. de, Souto, D.V. de O., Santos, H.E. da S., Silva, G.L.L. da, Athayde, A.C.R., 2012. Biological control of goat gastrointestinal helminthiasis by *Duddingtonia flagrans* in a semi-arid region of the northeastern Brazil. Vet. Parasitol. 188, 127–133. https://doi.org/10.1016/j.vetpar.2012.02.018

Vokřál, I., Michaela, Š., Radka, P., Jiří, L., Lukáš, P., Dominika, S., Kateřina, L., Barbora, S., Lenka, S., 2019. Ivermectin environmental impact: Excretion profile in sheep and phytotoxic effect in *Sinapis alba*. Ecotoxicol. Environ. Saf. 169, 944–949. https://doi.org/10.1016/j.ecoenv.2018.11.097

Waller, P.J., Faedo, M., Ellis, K., 2001a. The potential of nematophagous fungi to control the free-living stages of nematode parasites of sheep: towards the development of a fungal controlled release device. Vet. Parasitol. 102, 299–308.

Waller, P.J., Knox, M.R., Faedo, M., 2001b. The potential of nematophagous fungi to control the free-living stages of nematode parasites of sheep: feeding and block studies with *Duddingtonia flagrans*. Vet. Parasitol. 102, 321–330.

Waller, P.J., Ljungström, B.-L., Schwan, O., Martin, L.R., Morrison, D.A., Rydzik, A., 2006. Biological control of sheep parasites using *Duddingtonia flagrans*: Trials on commercial farms in Sweden. Acta Vet. Scand. 47, 23–32.

Wee, C.S., Ariff, M.S. Bin, Zakuan, N., Tajudin, M.N., 2012. Key Factors Affecting Consumer Purchase Intention a Study of Safe Vegetable in Ho Chi Minh City , Vietnam. Rev. Integr. Bus. Econ. Res. 3, 378–397.

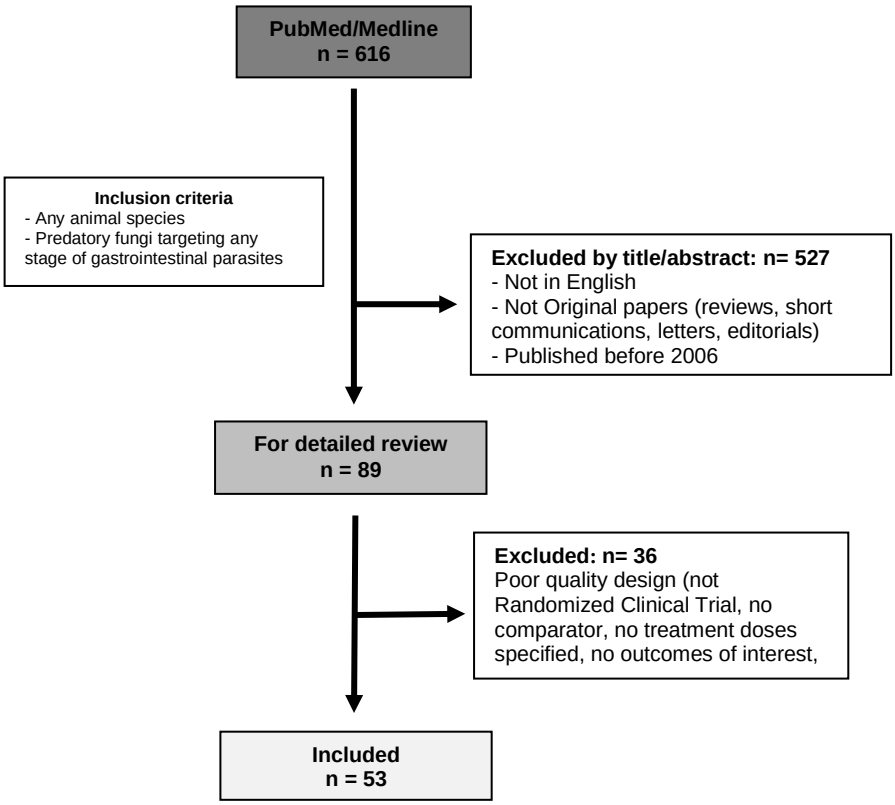

Figure 1- Flowchart with literature search.

Table 1 — PICO method for review question

Population	Gastrointestinal parasites of any animal species.
Intervention	Usage of nematophagous or predatory fungi on any stage of gastrointestinal parasites (e.g., eggs, larvae) in *in vivo*, *in vitro* and *ex vivo* settings. Only fungi that predate gastrointestinal parasites of animals were included.
Comparison	A control group was mandatory. Any relevant comparison was accepted (e.g. placebo, different fungi, different fungus dosage).
Outcome	Quantified control of gastrointestinal parasites (e.g. reduction of number of infective larvae in feces or pasture, reduction of number of eggs in feces or pasture, reduction of parasite load, reduction of deworming frequency). Only papers that quantified the outcomes with figures were included.

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769 Table 2 – Description of studies' characteristics

	Fungi	**Parasites**	**Administration and Dose**	**Frequency and duration**	**Evaluated Outcomes**
Ovine N = 15	*Duddingtonia flagrans* (15); *Monacrosporium thaumasium* (2); *Arthrobotrys robusta* (1); *Clonostachys rosea* (1)	*Haemonchus contortus* (14); *Trichostrongylus* spp. (8); *Teladorsagia* spp. (6) and others	$2x10^5 – 1x10^6$ chlamydospores /kg live weight (10); Other forms (fixed dose of *mycelia*, *conidia* and chlamydospores)	Single administration (2); Daily for 6 days to 5 months (10); Twice/week for 5 to 6 months (3)	Eggs per gram (EPG) reduction Fecal L3 reduction Reduction of intestinal adult parasites Reduction of L3 /kg pasture dry matter Reduction in Tracer Sheep parasite load
Bovines N = 10	*D. flagrans* (7); *Pochonia chlamydosporia* (2); *Arthrobotrys cladodes* (2); *M. thaumasium* (3); *A. robusta* (1)	*Cooperia* spp. (8); *H. contortus* (7); *Oesophagostomum* spp. (7) and others	0,2 – 0,25 g /10 kg live weight (4); Other forms (fixed dose of *mycelia* and chlamydospores)	Single administration (4); Daily for 10 days (1); Every 2 days for 30 days (1); Twice/week for 6 to 18 months (4)	EPG reduction Fecal L3 reduction Reduction of L3 /kg pasture dry matter
Equids N = 8	*D. flagrans* (7); *M. thaumasium* (3); *A. robusta* (1); *Mucor circinelloides* (2)	*Cyathostominae* (7), *Parascaris* sp. (1); *Strongyloides westeri* (1)	$1,5x10^5 – 2x10^6$ chlamydospores /kg live weight (3); Other forms (fixed dose of *mycelia* and *conidia*)	Single administration (3); Daily for 16 months (1); Twice/week for 21 days to 14 months (4)	EPG reduction Fecal L3 reduction Reduction of L3 /kg pasture dry matter Egg Reappearance Period after Ivermectin administration
Canids N = 7	*D. flagrans* (4); *Arthrobotrys* spp. (6); *Monacrosporium* spp. (6); *P. chlamydosporia* (2)	*Ancylostoma* spp. (3); *Angiostrongylus vasorum*. (2); *Toxocara canis* (2)	Mostly *in vitro*	Single administration (7)	Fecal L1 reduction Fecal L3 reduction Others

Caprine N = 5	*D. flagrans* (4); *Arthrobotrys* spp. (1)	*Trichostrongylus* spp. (5); *Haemonchus* spp. (4); *Teladorsagia* spp. (3) and others	$5x10^5$ – $1x10^6$ chlamydospores /kg live weight (4); 0,06g *mycelia* /kg live weight (1)	Single administration (1); Daily for 27 days to 5 months (3); Twice/week for 6 months (1)	EPG reduction Fecal L3 reduction Reduction of intestinal adult parasites Reduction of L3 /kg pasture dry matter Reduction in Tracer Goat parasite load
Swine N = 2	*D. flagrans* (2)	*Oesophagostomum* spp. (2)	Fixed dose of chlamydospores and mycelia (2); $5x10^5$ chlamydospores /kg live weight (1)	Single administration (2)	Fecal L3 reduction
Other N = 6	*D. flagrans* (5); *P. chlamydosporia* (2); *M. circinelloides* (2)	Varies with animal species	Varies with animal species	Single administration (4); Daily for 7 days (1); Every 2 days for 3 years (1)	EPG reduction Fecal L3 reduction Reduction of L3 /kg pasture dry matter Others

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771 N – Number of studies

772 Between brackets are the number of studies with each characteristic

773 EPG – Eggs per gram of feces

774 L1, L3 – parasite larval stages

***Conflict of Interest**

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: