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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, 21<sup>st</sup> June 2020

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

## 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

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

3

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25

26 **Abstract**

27 Background: Gastrointestinal parasites like nematodes are associated with significant  
28 impacts on animal health, causing poor growth rates, diseases and even death.  
29 Traditional parasite control includes the use of anthelmintic drugs, albeit being  
30 associated with drug resistance and ecotoxicity. In the last decade, biological control  
31 of parasites using nematophagous or predatory fungi has been increasingly studied,  
32 although systematic evidence of its efficacy is still lacking.

33  
34 The aim of this work was to assess the evidence of efficacy of nematophagous fungi  
35 in the control of nematodes and other gastrointestinal parasites in different animal  
36 species.

37  
38 Methods: Using the PICO method (Population, Intervention, Comparison and  
39 Outcomes), We-we performed a systematic review on the subject to search, applying  
40 the PICO method (Population, Intervention, Comparison and Outcomes), searching  
41 for original papers published between January 2006 and October 2019, written in  
42 English, and indexed in PubMed/Medline. Medical Subject Headings (MeSH) terms  
43 were used in the syntax. Papers were selected for detailed review based on title and  
44 abstract. Inclusion and exclusion criteria were applied, and relevant data ~~was~~-were  
45 collected from the remaining papers.

46  
47 Results: The literature search retrieved 616 papers. Eighty-nine were submitted to a  
48 detailed review. In the end, 53 papers were included in the analysis. The studies were  
49 very heterogeneous, using different fungi, doses, frequency of administration,  
50 duration of treatment, host animals, and target parasites. Considering the 53 papers,

51 44 studies (83% of the interventions) showed efficacy, with only 9 studies (17%)  
52 showing no significant differences when compared to control.

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

57

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

60

61

## 62 **Introduction**

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

67

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

76

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

93

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

100

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

107

108 | The aim of this study was to assess the evidence of efficacy ~~of using on the use of~~  
109 | nematophagous fungi in the control of nematodes in different animal species and in  
110 | various contexts, and also to present new insights on their efficacy against other  
111 | gastrointestinal parasites. The information gathered might be used in the future to  
112 | support recommendations and guidelines in the area of biological control of parasites.

113 | To the authors' best knowledge, this is the first systematic review regarding this  
114 | subject and covering several species of predatory fungi and hosts.

115

## 116 **Material & Methods**

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

121

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

126

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

130

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

138

### 139 **Results**

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

143

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

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151 2012; Bilotto et al., 2018; de Castro Oliveira et al., 2018; Dias et al., 2013; Luns et al.,  
152 2018; Mendoza-de Gives et al., 2018; Ortiz Pérez et al., 2017; Silva et al., 2013;  
153 Vieira et al., 2019), 8 on equids (Araujo et al., 2010; Braga et al., 2010; Braga et al.,  
154 2009a; Buzatti et al., 2015; Hernández et al., 2016, 2018; Tavela et al., 2011, 2013), 7  
155 on canids (Araujo et al., 2013; F. R. Braga et al., 2009b; Carvalho et al., 2009; de  
156 Freitas Soares et al., 2015; Maciel et al., 2009, 2010, 2012), 5 on caprine (Cai et al.,  
157 2017; Gómez-Rincón et al., 2007; Paraud et al., 2006, 2007; Vilela et al., 2012), 2 on  
158 swine (Facchini Rodrigues et al., 2018; Ferreira et al., 2011), 1 on hamster  
159 (Fernandes et al., 2012), 1 on feline (Braga et al., 2011), 1 on wild equids (Palomero  
160 et al., 2018), 1 hen (Hiura et al., 2015), and 2 papers with intervention in more than  
161 one animal species (Arias et al., 2013; Healey et al., 2018). Thirty studies were  
162 conducted in Brazil, 7 in Mexico, 5 in Spain, 2 in Australia, 2 in Argentina, 2 in  
163 France, 2 in the Netherlands, 1 in China, 1 in Switzerland and 1 in Sweden.

164

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

173

174 The fungal doses used varied between  $1.5 \times 10^5$  and  $5 \times 10^6$  chlamydozoospores/ kg of live  
175 weight, or between 0.1 and 0.25 grams of mycelia/ 10 kg of live weight, with the

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176 reductions in Eggs Per Gram and L3 varying between 0 and 100%. Only nine studies  
177 (17%) did not show significant differences between the intervention and control  
178 groups (six in sheep (Eysker et al., 2006a, 2006b; Faessler et al., 2007; Rocha et al.,  
179 2007; Silva et al., 2010; Waller et al., 2006), and the other three in horses, goats, and  
180 bovines (Buzatti et al., 2015; Mendoza-de Gives et al., 2018; Paraud et al., 2007)).  
181 The explanations for poor results vary, and these could be dependent on low  
182 temperatures and initial overall low parasite loads.

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

192

### 193 **Discussion**

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

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

203

204 In total, 17% of the studies did not show significant differences between treated and  
205 control groups. Explanations for the negative results are debatable. Some authors  
206 argued that the dilution of spores due to ~~a~~ greater food consumption by lactating  
207 animals might explain the poor parasite reduction (Eysker et al., 2006b). Others  
208 defended that the low temperatures during the trials compromised fungal growth  
209 (Paraud et al., 2007). ~~Finally, while~~ others found no significant efficacy comparing to  
210 the control group because the good health of the animals and the parasitological  
211 programs already in place prevented the fungal activity to stand out (Waller et al.,  
212 2006). However, the range of fungal doses used ~~were-was~~ not pointed as a reasons for  
213 the negative results.

214

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

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

231

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

239

240 Fitz-Aranda et al. (2015) stated that some of the biggest factors influencing the  
241 success of this kind of treatment are the medium in which the fungi are administered,  
242 the ingestion of an effective dose, as well as a storage method that does not alter the  
243 fungi's properties. Mixing spores into a nutrient block is an approach already tested,  
244 but it has shown the downside of offering a very variable spore intake among animals  
245 in the same group, with the ingestion also depending on climate and grazing season  
246 (Sagüés et al., 2011). Additionally, the blocks have a relatively high level of moisture  
247 comparing to other forms of administration, reducing the product's shelf life (Larsen,  
248 2006). The use of cereal grains combined with chlamydo spores has similarly shown  
249 to be effective (Facchini Rodrigues et al., 2018; Waller et al., 2001b).

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251 The use of intraruminal Controlled Release Devices has demonstrated good results in  
252 ruminants (Sagüés et al., 2014) and presents itself as a good option for future  
253 formulations, especially for free-ranging animals (Waller et al., 2001a). Buzatti et al.  
254 (2015) showed that administrations with 3-day intervals in horses are effective in  
255 reducing the amount of fecal cyathostomin L3, which is also a good prospect for  
256 extensive grazers.

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257

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

267

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

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

281

282 It has been suggested that abiotic factors like rain, temperature, and sunlight might  
283 influence the performance of the nematophagous fungi. Field trials performed with *D.*  
284 *flagrans* in horses, registered better results in Spring/Summer, than in  
285 Autumn/Winter, concerning L3 reduction levels in fecal samples and pasture  
286 (Madeira de Carvalho et al., 2007). Indeed, several studies show different fungal  
287 efficacies depending on ~~the ambient~~ temperature. Paraud et al. (2006) obtained higher  
288 reductions of *H. contortus*, *T. circumcincta* and *T. colubriformis* by *D. flagrans* at  
289 21°C than at 28°C. Also, Bilotto et al. (2018) achieved 63.77% and 88.39% L3  
290 reduction in pasture in sunny and shaded conditions in summer, respectively, versus  
291 1,58% and 3.56% in winter. This shows that the relation between temperature, larval  
292 development, and fungal efficacy needs to be understood, in order to design  
293 successful parasite-management programs.

294

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

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

307

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

313

314 From the 53 analyzed articles, only six (11.3%) (Assis et al., 2012, 2013; Dias et al.,  
315 2013; Hernández et al., 2016, 2018; Palomero et al., 2018) reported data regarding the  
316 administration of fungi for 12 or more months. This is elucidative of the lack of  
317 studies that measure the long-term impact of nematophagous fungi on a parasite  
318 population. All these studies showed a good long-term reduction of EPG, whether by  
319 using ovicidal, larvicidal, or a mixture of both types of fungi. One short-term study  
320 (Thapa et al., 2018) has shown an unwanted effect when using *P. chlamydosporia*  
321 (ovicidal) against an ascarid parasite of chickens: the reduced parasite burden caused  
322 by the exposure to *P. chlamydosporia* led to ~~a~~ faster development of the existing  
323 larvae into adult worms within the chicken's organismintestine. That, in turn meant a  
324 higher EPG and a greater contamination of the environment. This is an extremely  
325 important point to have in consideration when designing parasite control programs,

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

329

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

336

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

348

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

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

357 |  
358 | The fungal doses used varied widely, but no significant correlation between low  
359 | fungal intake and poor outcomes was found with the doses studied. Although several  
360 | regimens of fungal administration have been tested and overall good results have been  
361 | achieved, optimal parasite control is expected to be attained when adopting long term  
362 | schedules, with at least a twice-per-week fungal administration, as showed by Assis et  
363 | al. (2013), Hernández et al. (2016) and Silva et al. (2009).

364 |  
365 | In general, papers with negative or absent results tend to be less published, which can  
366 | limit the information retrieved by systematic reviews. Despite all the advances in  
367 | understanding predatory fungi, some questions remain unaddressed. There is scarce  
368 | information about the fungi's potential impact on the soil microbiome. The fungi's  
369 | attack mechanisms are based on physical and chemical actions simpler than those of  
370 | anthelmintic drugs. This could be associated with a decreased rate of development of  
371 | parasite resistance to predatory fungi. Likewise, there is a need to further evaluate the  
372 | possibility of predatory fungi infecting immunocompromised humans, limiting their  
373 | potential use. Consequently, this will continue to be a hot topic within the veterinarian  
374 | community for the coming years. But it will also be a vanguard tool for animal  
375 | parasite control with great potential in the future, namely because it is an ecological,

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

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379

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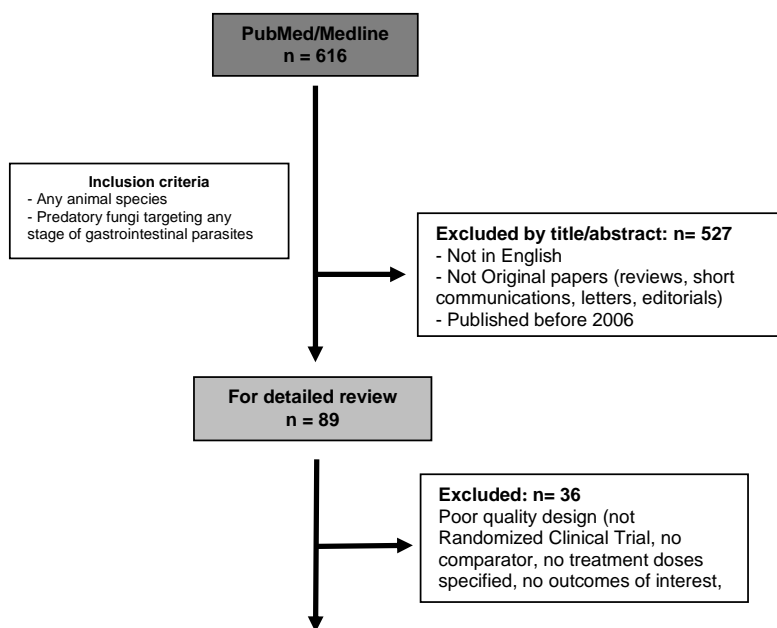
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754 Figure 1- Flowchart with literature search.

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767 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 <i>in vivo</i> , <i>in vitro</i> and <i>ex vivo</i> 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
<b>Ovine</b> <b>N = 15</b>	<i>Duddingtonia flagrans</i> (15); <i>Monacrosporium thaumasium</i> (2); <i>Arthrotrys robusta</i> (1); <i>Clonostachys rosea</i> (1)	<i>Haemonchus contortus</i> (14); <i>Trichostrongylus</i> spp. (8); <i>Teladorsagia</i> spp. (6) and others	2x10 <sup>5</sup> – 1x10 <sup>6</sup> chlamydo-spores /kg live weight (10); Other forms (fixed dose of <i>mycelia</i> , <i>conidia</i> and chlamydo-spores)	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
<b>Bovines</b> <b>N = 10</b>	<i>D. flagrans</i> (7); <i>Pochonia</i>	<i>Cooperia</i> spp. (8); <i>H. contortus</i> (7);	0,2 – 0,25 g /10 kg live weight (4);	Single administration	EPG reduction Fecal L3 reduction

	<i>chlamydosporea</i> (2); <i>Arthrotrys cladodes</i> (2); <i>M. thaumasium</i> (3); <i>A. robusta</i> (1)	<i>Oesophagostomum</i> spp. (7) and others	Other forms (fixed dose of <i>mycelia</i> and <i>chlamydospores</i> )	(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
<b>Equids</b> N = 8	<i>D. flagrans</i> (7); <i>M. thaumasium</i> (3); <i>A. robusta</i> (1); <i>Mucor circinelloides</i> (2)	<i>Cyathostominae</i> (7), <i>Parascaris</i> sp. (1); <i>Strongyloides westeri</i> (1)	1,5x10 <sup>5</sup> – 2x10 <sup>6</sup> <i>chlamydospores</i> /kg live weight (3); Other forms (fixed dose of <i>mycelia</i> and <i>conidia</i> )	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
<b>Canids</b> N = 7	<i>D. flagrans</i> (4); <i>Arthrotrys</i> spp. (6); <i>Monacrosporium</i> spp. (6); <i>P. chlamydosporea</i> (2)	<i>Ancylostoma</i> spp. (3); <i>Angiostrongylus vasorum</i> . (2); <i>Toxocara canis</i> (2)	Mostly <i>in vitro</i>	Single administration (7)	Fecal L1 reduction Fecal L3 reduction Others
<b>Caprine</b> N = 5	<i>D. flagrans</i> (4); <i>Arthrotrys</i> spp. (1)	<i>Trichostrongylus</i> spp. (5); <i>Haemonchus</i> spp. (4); <i>Teladorsagia</i> spp. (3) and others	5x10 <sup>5</sup> – 1x10 <sup>6</sup> <i>chlamydospores</i> /kg live weight (4); 0,06g <i>mycelia</i> /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
<b>Swine</b> N = 2	<i>D. flagrans</i> (2)	<i>Oesophagostomum</i> spp. (2)	Fixed dose of <i>chlamydospores</i> and <i>mycelia</i> (2); 5x10 <sup>5</sup> <i>chlamydospores</i> /kg live weight (1)	Single administration (2)	Fecal L3 reduction
<b>Other</b> N = 6	<i>D. flagrans</i> (5); <i>P. chlamydosporea</i> (2); <i>M. circinelloides</i> (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

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

3

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26 **Abstract**

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

35

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

43

44 Results: The literature search retrieved 616 papers. Eighty-nine were submitted to a  
45 detailed review. In the end, 53 papers were included in the analysis. The studies were  
46 very heterogeneous, using different fungi, doses, frequency of administration,  
47 duration of treatment, host animals, and target parasites. Considering the 53 papers,  
48 44 studies (83% of the interventions) showed efficacy, with only 9 studies (17%)  
49 showing no significant differences when compared to control.

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

54

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

57

58

## 59 **Introduction**

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

64

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

73

74 This situation has stimulated research in strategies based on natural approaches

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

90

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

97

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

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

104

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

112

### 113 **Material & Methods**

114 The systematic review comprised original papers published between January 2006  
115 and October 2019. Title and abstract selection were performed in accordance with the  
116 PICO strategy and the review question was defined by the PICO components (Table  
117 1).

118

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

123

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

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

127

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

134

## 135 **Results**

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

139

140 In Table 2, the studies' characteristics regarding animal species, fungi used, parasites  
141 targeted, dose, frequency, duration and form of administration and evaluated  
142 outcomes are detailed. Accordingly, a total of 15 intervention studies were reported  
143 on ovine (Aguilar-Marcelino et al., 2016; Aguilar et al., 2008; Da Silva et al., 2015;  
144 Eysker et al., 2006a, 2006b; Faessler et al., 2007; Gómez-Rincón et al., 2006; Kahn et  
145 al., 2007; Mendoza-De-Gives et al., 2006; Rocha et al., 2007; Sagüés et al., 2011;  
146 Silva et al., 2009, 2010, 2011; Waller et al., 2006), 10 on bovines (Assis et al., 2013,  
147 2012; Bilotto et al., 2018; de Castro Oliveira et al., 2018; Dias et al., 2013; Luns et al.,  
148 2018; Mendoza-de Gives et al., 2018; Ortiz Pérez et al., 2017; Silva et al., 2013;  
149 Vieira et al., 2019), 8 on equids (Araujo et al., 2010; Braga et al., 2010; Braga et al.,

150 2009a; Buzatti et al., 2015; Hernández et al., 2016, 2018; Tavela et al., 2011, 2013), 7  
151 on canids (Araujo et al., 2013; F. R. Braga et al., 2009b; Carvalho et al., 2009; de  
152 Freitas Soares et al., 2015; Maciel et al., 2009, 2010, 2012), 5 on caprine (Cai et al.,  
153 2017; Gómez-Rincón et al., 2007; Paraud et al., 2006, 2007; Vilela et al., 2012), 2 on  
154 swine (Facchini Rodrigues et al., 2018; Ferreira et al., 2011), 1 on hamster  
155 (Fernandes et al., 2012), 1 on feline (Braga et al., 2011), 1 on wild equids (Palomero  
156 et al., 2018), 1 hen (Hiura et al., 2015), and 2 papers with intervention in more than  
157 one animal species (Arias et al., 2013; Healey et al., 2018). Thirty studies were  
158 conducted in Brazil, 7 in Mexico, 5 in Spain, 2 in Australia, 2 in Argentina, 2 in  
159 France, 2 in the Netherlands, 1 in China, 1 in Switzerland and 1 in Sweden.

160

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

169

170 The fungal doses used varied between  $1.5 \times 10^5$  and  $5 \times 10^6$  chlamydozoospores/ kg of live  
171 weight, or between 0.1 and 0.25 grams of mycelia/ 10 kg of live weight, with the  
172 reductions in Eggs Per Gram and L3 varying between 0 and 100%. Only nine studies  
173 (17%) did not show significant differences between the intervention and control  
174 groups (six in sheep (Eysker et al., 2006a, 2006b; Faessler et al., 2007; Rocha et al.,

175 2007; Silva et al., 2010; Waller et al., 2006), and the other three in horses, goats, and  
176 bovines (Buzatti et al., 2015; Mendoza-de Gives et al., 2018; Paraud et al., 2007)).  
177 The explanations for poor results vary, and these could be dependent on low  
178 temperatures and initial overall low parasite loads.

179

180 A total of 44 studies (83%) demonstrated efficacy. In the sheep studies, 100%  
181 targeted Trichostrongylidae and 13% Chabertidae. In bovines, Trichostrongylidae  
182 were targeted in 90% of the studies and Chabertidae in 80%. Strongylidae were  
183 targeted in 87,5% of the horse studies, Cyathostominae in 75% and Ascarididae in  
184 12,5%. In the dog studies, the most targeted parasites were Ancylostomatidae (43%),  
185 followed by Angiostrongylidae and Ascarididae (29% each). In studies with goats,  
186 100% were targeted to Trichostrongylidae and 20% to Chabertidae. More details can  
187 be found in Supplementary Tables 1 – 7.

188

## 189 **Discussion**

190 Systematic reviews are the best way to properly collect and gather data, in order to  
191 retrieve the strongest evidence from the literature (Sargeant and O'Connor, 2014).

192 The current review confirmed that treatments with nematophagous fungi reduce the  
193 number and viability of gastrointestinal parasites' larvae and eggs, both *in vivo* and *in*  
194 *vitro* experiments, in distinct animal species. The reviewed papers showed very  
195 satisfactory results in reducing the need for frequent use of anthelmintic drugs.

196 Authors also reported an increase in time between deworming when using *D. flagrans*  
197 and *M. circinelloides* in horses (Hernández et al., 2016, 2018) and wild equids  
198 (Palomero et al., 2018).

199

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

210

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

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

227

228 Besides the already tested effect of *D. flagrans* on L3 of gastrointestinal nematodes,  
229 there are reports of its predatory activity against *Angiostrongylus vasorum* first-stage  
230 larvae (L1) (Braga et al., 2009b) and *Toxocara canis* second-stage larvae (L2) (Hiura  
231 et al., 2015). Using crude extracts from *M. thaumasium*, de Freitas Soares et al.  
232 (2015) have shown a reduction of *A. vasorum* L1 *in vitro*. This is a new approach to  
233 parasite control, namely for pets, that deserves further investigation.

234

235 Fitz-Aranda et al. (2015) stated that some of the biggest factors influencing the  
236 success of this kind of treatment are the medium in which the fungi are administered,  
237 the ingestion of an effective dose, as well as a storage method that does not alter the  
238 fungi's properties. Mixing spores into a nutrient block is an approach already tested,  
239 but it has shown the downside of offering a very variable spore intake among animals  
240 in the same group, with the ingestion also depending on climate and grazing season  
241 (Sagüés et al., 2011). Additionally, the blocks have a relatively high level of moisture  
242 comparing to other forms of administration, reducing the product's shelf life (Larsen,  
243 2006). The use of cereal grains combined with chlamydospores has similarly shown  
244 to be effective (Facchini Rodrigues et al., 2018; Waller et al., 2001b).

245

246 The use of intraruminal Controlled Release Devices has demonstrated good results in  
247 ruminants (Sagüés et al., 2014) and presents itself as a good option for future  
248 formulations, especially for free-ranging animals (Waller et al., 2001a). Buzatti et al.  
249 (2015) showed that administrations with 3-day intervals in horses are effective in

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

252

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

262

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

275

276 It has been suggested that abiotic factors like rain, temperature, and sunlight might  
277 influence the performance of the nematophagous fungi. Field trials performed with *D.*  
278 *flagrans* in horses, registered better results in Spring/Summer, than in  
279 Autumn/Winter, concerning L3 reduction levels in fecal samples and pasture  
280 (Madeira de Carvalho et al., 2007). Indeed, several studies show different fungal  
281 efficacies depending on ambient temperature. Paraud et al. (2006) obtained higher  
282 reductions of *H. contortus*, *T. circumcincta* and *T. colubriformis* by *D. flagrans* at  
283 21°C than at 28°C. Also, Bilotto et al. (2018) achieved 63.77% and 88.39% L3  
284 reduction in pasture in sunny and shaded conditions in summer, respectively, versus  
285 1,58% and 3.56% in winter. This shows that the relation between temperature, larval  
286 development, and fungal efficacy needs to be understood, in order to design  
287 successful parasite-management programs.

288

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

300

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

306

307 From the 53 analyzed articles, only six (11.3%) (Assis et al., 2012, 2013; Dias et al.,  
308 2013; Hernández et al., 2016, 2018; Palomero et al., 2018) reported data regarding the  
309 administration of fungi for 12 or more months. This is elucidative of the lack of  
310 studies that measure the long-term impact of nematophagous fungi on a parasite  
311 population. All these studies showed a good long-term reduction of EPG, whether by  
312 using ovicidal, larvicidal, or a mixture of both types of fungi. One short-term study  
313 (Thapa et al., 2018) has shown an unwanted effect when using *P. chlamydosporia*  
314 (ovicidal) against an ascarid parasite of chickens: the reduced parasite burden caused  
315 by the exposure to *P. chlamydosporia* led to faster development of the existing larvae  
316 into adult worms within the chicken's intestine. That, in turn meant a higher EPG and  
317 a greater contamination of the environment. This is an extremely important point to  
318 have in consideration when designing parasite control programs, since it may increase  
319 the possibility of environment recontamination. More studies need to be conducted in  
320 order to fully understand how beneficial these treatments are in the long-term.

321

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

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

327

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

339

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

346

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

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

353

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

367

368

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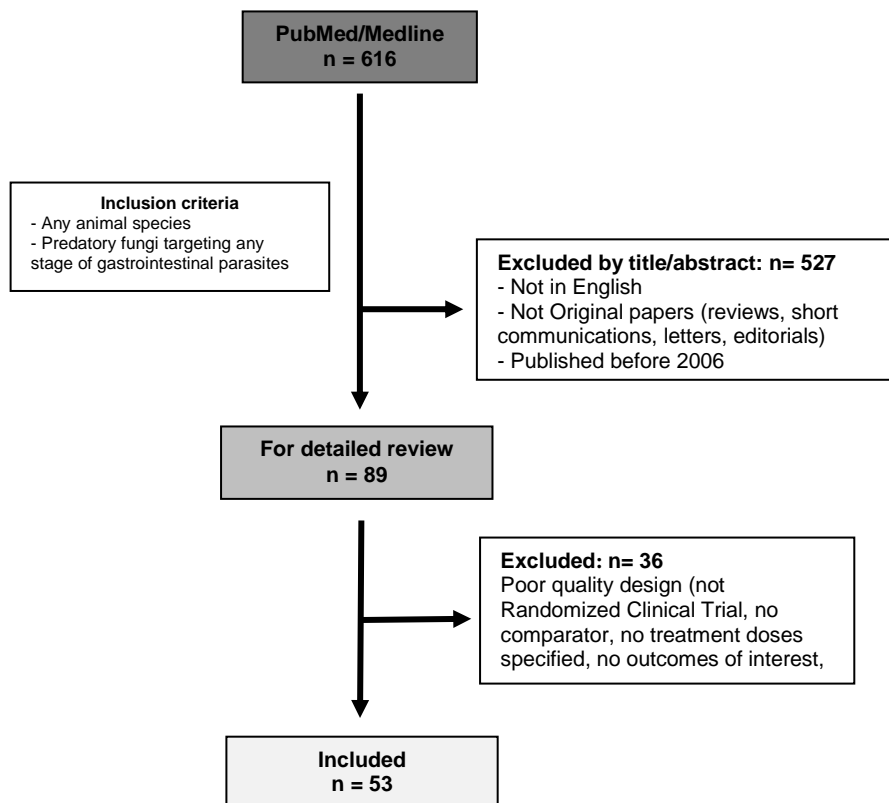


Figure 1- Flowchart with literature search.

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756 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 <i>in vivo</i> , <i>in vitro</i> and <i>ex vivo</i> 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

	<b>Fungi</b>	<b>Parasites</b>	<b>Administration and Dose</b>	<b>Frequency and duration</b>	<b>Evaluated Outcomes</b>
<b>Ovine</b> <i>N = 15</i>	<i>Duddingtonia flagrans</i> (15); <i>Monacrosporium thaumasium</i> (2); <i>Arthrobotrys robusta</i> (1); <i>Clonostachys rosea</i> (1)	<i>Haemonchus contortus</i> (14); <i>Trichostrongylus</i> spp. (8); <i>Teladorsagia</i> spp. (6) and others	$2 \times 10^5 - 1 \times 10^6$ chlamydo-spores /kg live weight (10); Other forms (fixed dose of <i>mycelia</i> , <i>conidia</i> and chlamydo-spores)	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
<b>Bovines</b> <i>N = 10</i>	<i>D. flagrans</i> (7); <i>Pochonia chlamydo-sporea</i> (2); <i>Arthrobotrys cladodes</i> (2); <i>M. thaumasium</i> (3); <i>A. robusta</i> (1)	<i>Cooperia</i> spp. (8); <i>H. contortus</i> (7); <i>Oesophagostomum</i> spp. (7) and others	0,2 – 0,25 g /10 kg live weight (4); Other forms (fixed dose of <i>mycelia</i> and chlamydo-spores)	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
<b>Equids</b> <i>N = 8</i>	<i>D. flagrans</i> (7); <i>M. thaumasium</i> (3); <i>A. robusta</i> (1); <i>Mucor circinelloides</i> (2)	<i>Cyathostominae</i> (7), <i>Parascaris</i> sp. (1); <i>Strongyloides westeri</i> (1)	$1,5 \times 10^5 - 2 \times 10^6$ chlamydo-spores /kg live weight (3); Other forms (fixed dose of <i>mycelia</i> and <i>conidia</i> )	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
<b>Canids</b> <i>N = 7</i>	<i>D. flagrans</i> (4); <i>Arthrobotrys</i> spp. (6); <i>Monacrosporium</i> spp. (6); <i>P. chlamydo-sporea</i> (2)	<i>Ancylostoma</i> spp. (3); <i>Angiostrongylus vasorum</i> . (2); <i>Toxocara canis</i> (2)	Mostly <i>in vitro</i>	Single administration (7)	Fecal L1 reduction Fecal L3 reduction Others

<b>Caprine</b> N = 5	<i>D. flagrans</i> (4); <i>Arthrobotrys</i> spp. (1)	<i>Trichostrongylus</i> spp. (5); <i>Haemonchus</i> spp. (4); <i>Teladorsagia</i> spp. (3) and others	$5 \times 10^5 - 1 \times 10^6$ chlamydozooids /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
<b>Swine</b> N = 2	<i>D. flagrans</i> (2)	<i>Oesophagostomum</i> spp. (2)	Fixed dose of chlamydozooids and mycelia (2); $5 \times 10^5$ chlamydozooids /kg live weight (1)	Single administration (2)	Fecal L3 reduction
<b>Other</b> N = 6	<i>D. flagrans</i> (5); <i>P.</i> <i>chlamydozooidia</i> (2); <i>M.</i> <i>circinelloides</i> (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

**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: