Review

Biocontrol of Avian Gastrointestinal Parasites Using Predatory Fungi: Current Status, Challenges, and Opportunities

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Abstract: This review describes the current research status regarding the implementation of predatory fungi in the biological control approach of bird gastrointestinal (GI) parasitosis. The main GI parasites of Galliformes (e.g., broilers, layers, peacocks, pheasants) and Ratites (e.g., ostriches, emus, rheas) are addressed, as well as their impact on farms, zoos, and private collections. The main characteristics regarding biocontrol with predatory fungi are briefly described, such as their mode of action and efficacy against GI parasites of different animal hosts. The state of the art regarding the use of predatory fungi in birds is reviewed here by describing all associated articles already published in the main databases, techniques, and their main findings. Ovicidal fungi such as Pochonia chlamydosporia, Metarhizium spp. and Acremonium spp., and larvicidal fungi, namely Duddingtonia flagrans, Arthrobotrys spp. and Monacrosporium thaumasium, have shown promising predacious activity against ascarid eggs and nematode larvae from chickens and ostriches, both in vitro and in vivo, also revealing tolerance to the GI passage in chickens and maintenance of predacious capacity. Further studies are needed to understand the fungi–parasite–host gut microbiota interactions and target other avian GI parasitic species, such as nematodes, coccidia, cestodes, and trematodes.

Keywords: birds; intestinal parasites; biological control; predatory fungi

1. Gastrointestinal Parasites of Galliformes and Ratites

Domestic and exotic birds are commonly exposed to a wide variety of generalist or host-specific gastrointestinal (GI) parasites, with different life cycles and levels of pathogenicity [1-8].

In Galliformes kept on free-range farms, zoos, and public gardens (e.g., broilers, layers, peacocks, pheasants), coccidial infections caused by Eimeria spp. and Isospora spp. can reach prevalence and shedding values up to 80% and 15,000 oocysts per gram of feces (OPG), respectively [6,9-14] and are currently responsible for average losses of approximately 12 billion € annually worldwide in the poultry industry [5,15]. Nematode infections are also a serious problem in Galliformes, being ascarids (e.g., Ascaridia galli), heterakids (e.g., Heterakis gallinarum and H. isolonche), capillarids (e.g., Capillaria spp.), strongyles (e.g., Trichostrongylus tenuis), and Strongyloides spp., the most frequent and pathogenic species [6,8-11,13,16,17].

Larger birds like Ratites (e.g., ostriches, emus and rheas), which are commonly kept in zoos worldwide for ornamental exhibition and occasionally in farms for production purposes, are also susceptible to GI parasitic infections, and nematodes belonging to...
the genera *Libyostrongylus* and *Codiostomum* are of most clinical importance, especially *Libyostrongylus douglassii*, which is responsible for the rotten stomach disease [6,18–22].

The control of these agents based solely on the administration of antiparasitic compounds (e.g., anticoccidials and anthelmintics) is of limited utility, since they do not act on the environmental forms of the parasites. In addition, common drug misuse in livestock farms often leads to efficacies lower than expected, appearance of drug resistance, and potential contamination of the environment with drug residues [23–26].

New complementary strategies are being proposed for integrated GI parasite control in domestic and wild animals kept in captivity, namely the use of predatory fungi as an accurate, innovative, natural, and sustainable tool [27,28].

2. Biocontrol of GI Parasites Using Predatory Fungi

Over the past 20 years, there has been an increasing interest in research regarding the use of predatory fungi (also referred as “nematophagous fungi”, or more recently “helminthophagous fungi”) for the biocontrol of animal gastrointestinal parasites, in complement with drug treatments.

These are saprophytic filamentous fungi belonging mainly to the phyla Ascomycota and Mucoromycota, often found in agricultural soil and organic decaying matter, which play a role in the recycling of carbon, nitrogen, and other elements originating from nematode degradation [29]. Besides their common saprophytic characteristics, these fungi also have the ability to predate intestinal parasites of animals, especially the eggs and larvae, which serve as an additional source of nutrients for fungal growth. Their tolerance to the animal’s gastrointestinal transit has already been demonstrated, being expelled with feces to the soil, where they start predating parasitic forms, especially in micro-fecal and peri-fecal environments [30].

There are three main groups of predatory fungi, defined according to their mode of action: larvicidal, ovicidal, and endoparasitic, the first two being the most commonly used in biocontrol trials. For larvicidal fungi such as *Duddingtonia flagrans*, *Arthrobotrys* spp., and *Monacrosporium thauamatum*, the main feature is the production of a wide diversity of traps (e.g., constricting rings, non-constricting rings, adhesive nodules, and ramifications), whose formation is stimulated by the presence of helminth larvae. For ovicidal fungi, namely *Mucor circinelloides*, *Pochonia chlamydosporia*, *Verticillium* spp., *Purpureocillium lilacinum* (formerly known as *Paecilomyces lilacinus*) and *Trichoderma* spp., the main characteristic consists of their ability to predate helminth eggs, and it is the presence of parasite eggs that triggers fungal hyphae migration towards their cuticula, in which mechanic and enzymatic activity are developed [29].

Both larvicidal and ovicidal fungi have been used in several in vitro and in vivo experiments, being unanimously considered an accurate and sustainable tool for the control of GI parasites, resulting in a reduction in the number of eggs per gram of feces (EPG) of 60–97% in field trials with grazing animals [28,31–35]. The lack of adverse effects of *D. flagrans* on soil nematodes [36], as well as the innocuousness of *M. circinelloides* and *D. flagrans* on several animal species [35,37] should also be underlined.

These fungi have already been isolated in America [38–42], Europe [43], Asia [44,45], Oceania [46,47], and even in Antarctica [48], and two commercial formulations of *D. flagrans* are already commercially available in Australia and New Zealand (BioWorma®—NCIMB 30336, BioWorma, Sydney, Australia) and in Brazil (Bioverm®—AC001, GhenVet Saúde Animal, Paulínia, Brazil).

3. Testing the Use of Predatory Fungi against Avian GI Parasites: State of the Art

Despite the increasing number of studies in this topic, most of them are focused on the biocontrol of intestinal parasites affecting ruminants and horses, and there is a lack of research regarding the use of predatory fungi in other animals, such as birds.

A literature search was performed in November 2021, in PubMed, Scopus, Web of Science and Google Scholar databases, using the search string “(predatory fungi OR
predacious fungi OR duddingtonia OR arthrobotrys OR monacrosporium OR mucor OR pochonia OR verticillium OR paecilomyces OR trichoderma) AND (coccidia OR helminth OR nematode)." Title and abstract analysis were performed, only research articles in English and published from 1990 until 2021 were included, and other types of publications (e.g., reviews, letters, and editorials) were excluded. It was found that only 5 publications were related to in vitro and in vivo experiments using predatory fungi against avian GI parasites (4 original research articles and 1 research note), carried out in Brazil and Denmark (Table 1).

Table 1. In vitro and in vivo research performed with predatory fungi against avian GI parasites.

<table>
<thead>
<tr>
<th>Type of Assay</th>
<th>Fungal Species (Biotype)</th>
<th>Target Organism</th>
<th>Study Objectives</th>
<th>Reference</th>
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<tbody>
<tr>
<td>In vitro</td>
<td><em>D. flagrans</em> (AC001; CG722) <em>A. cladodes</em> (CG719)</td>
<td><em>L. douglassii</em></td>
<td>Test larvicidal activity against L3 larvae</td>
<td>[49]</td>
</tr>
<tr>
<td></td>
<td><em>P. chlamydospora</em> (Biotype 10) <em>Me. brunneum</em> (KVL04-57; KVL16-26) <em>Me. carneum</em> (KVL16-33) <em>Acremonium</em> sp. (KVL16-34)</td>
<td><em>A. galli</em> <em>H. gallinarum</em></td>
<td>Test ovicidal activity in different soil types; isolate native ovicidal fungi</td>
<td>[50]</td>
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<tr>
<td></td>
<td><em>D. flagrans</em> (AC001; CG722) <em>M. thamnium</em> (NF34A)</td>
<td><em>Panagrellus</em> spp.</td>
<td>Test GI passage in chickens and evaluate the maintenance of germination and larvicidal capacities</td>
<td>[51]</td>
</tr>
<tr>
<td>In vivo</td>
<td><em>P. chlamydospora</em> (VC4)</td>
<td><em>A. galli</em> <em>H. gallinarum</em></td>
<td>Test ovicidal activity in different soil types; evaluate the interaction soil-fungi in birds worm population and burdens, and egg counting</td>
<td>[52]</td>
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The first in vitro experiment with predatory fungi against avian intestinal parasites was reported 9 years ago by Braga et al. [49]. The study aimed to test the larvicidal activity of two isolates of *D. flagrans* (AC001 and CG722) and one isolate of *Arthrobotrys cladodes* (CG719) on infective larvae (L3) of *L. douglassii*. The assays were performed in plates with Water-Agar medium (WA, 2%) and the number of non-preyed L3 was counted daily, for seven days of incubation, in all treated and control groups. Percentage reductions of L3 were found to be significant between test and control plates, totalizing efficacies of 85.2% (isolate AC001), 81.2% (CG722), and 89.2% (CG719). Isolates did not differ in the daily mean of non-preyed L3, but all of them differed significantly from control plates, and therefore these isolates offer potential to be used in the biocontrol of GI nematodes of ratites.

Another in vitro study was conducted in Denmark by Thapa et al. [50], which aimed to test the performance of *P. chlamydospora* (Biotype 10) and *Metarhizium brunneum* (KVL04-57) against non-embryonated ascarid eggs (*A. galli* and *Heterakis* spp.) in sterilized and non-sterilized soils. Egg recovery was examined before and after incubation at 22 °C for 30 days. In sterilized soil, results were significantly influenced by the interaction between fungal treatment and incubation time, with egg count differing between treatments and controls after 30 days of incubation, and *P. chlamydospora* and *Me. brunneum* showing reduction efficacies of 46% and 30%, respectively. However, in non-sterilized soil, the outcomes were slightly different, with both fungal and control plates showing significant egg recovery reductions (68–77%). In this case, only *Me. brunneum* treatment resulted in slight but significant reductions in comparison with controls and *P. chlamydospora* plates. These results suggest that resource competition between predatory fungi and native soil microbiota may interfere negatively with the performance of fungal isolates, as well as
rejects the hypothesis of potential environmental impact on soil microbiota caused by the administration of these fungi.

In this study, the authors also aimed to evaluate the survival of ascarid eggs in different soil types, both in sterilized and non-sterilized soil, after 30 days of incubation at 22 °C. For sterilized soils, only incubation time and soil type had a significant interaction on egg recovery. For non-sterilized soils, the egg counts were significantly reduced in all soil types, ranging from 38% to 99%. Non-sterilized soils exhibiting the highest ovicidal activities were also used to isolate, identify, and test the antagonistic effect of native fungi against ascarid eggs. Fungal isolates belonged to the genera *Metarhizium* and *Acremonium*; however, none of the three isolates revealed predatory efficacies higher than 34% after 28 days of exposure. These results also suggest that soil has inherent biotic egg-degrading properties, namely due to its native microbiota.

Predatory fungi have also been tested in vivo in chickens and hens, with the first published report dating back to 2017. The study developed by Silva et al. [51] aimed to test the maintenance of germination and larvicidal capacities of *D. flagrans* (AC001; CG722) and *M. thaumastium* (NF34A) after passing through the GI tract of chickens. For this purpose, four experimental groups with two chickens were considered: three groups were provided with autoclaved concentrate feed mixed with 1 mL of an aqueous solution containing $6.4 \times 10^4$ spores of each isolate (test groups), and 1 group received feed mixed with distilled water (control group), on a daily basis. Fecal samples were collected 6, 12, 24, 48, and 72 h post-administration, and placed in Petri dishes with WA medium. Suspensions containing larvae of the free-living nematode *Panagrellus* spp. were also added to each plate, followed by incubation at 25 °C for 12 days, to test mycelial growth and average number of recovered larvae in each period of administration. Fungal structures from all isolates were observed at 6, 12, and 24 h post-administration, confirming the ability of spores to resist the GI passage in chickens. In addition, the highest percentage of reduction in the number of recovered larvae was identified at 6 h post-administration, averaging reduction rates of approximately 35% to 71%, with only isolate AC001 showing a significant reduction in comparison with the control plates. Despite larvicidal activity being tested against free-living nematodes, results from this study can be extrapolated to parasitic nematodes affecting bird species, due to a similar mode of action.

A study conducted by Valadão et al. [52] also aimed to test the maintenance of germination and ovicidal capacities of *P. chlamydosporia* (VC4) after GI transit in chickens, with an experimental design similar to the previously mentioned study. A group of 22 chickens was divided into two experimental groups: both groups received a supplementation of shredded corn for 7 days, after which only the test group started to receive the supplement inoculated with *P. chlamydosporia*. Samples were collected in each group after 0, 6, 8, 10, 12, 18, and 24 h post-administration, and placed in plates with WA medium, followed by incubation at 25 °C for 30 days, to check for the growth of *P. chlamydosporia*. The authors reported the identification of VC4 isolate only in samples from the test group, and 6 h post administration. VC4 isolates obtained after 30 days of incubation were used for further in vitro tests in WA medium, aiming to check the maintenance of ovicidal activity against *A. galli* and *H. gallinarum* eggs. A significant reduction in egg viability was observed after 74 h of incubation and the highest rates were recorded after 144 h, totalizing approximately 60% and 40% for *A. galli* and *H. gallinarum*, respectively.

Finally, a study performed by Thapa et al. [53] aimed to evaluate the performance of *P. chlamydosporia* (Biotype 10) in reducing worm burden and ascarid egg count in hens, by jointly giving the fungus with sterilized and non-sterilized soil. These soils were previously used in in vitro trials aiming to evaluate the egg recovery in sterilized and non-sterilized substrates inoculated with *P. chlamydosporia*. For the in vivo trial, birds were fed with the same soils together with the morning meal, comprising four experimental groups: sterilized control soil (SC), sterilized soil with fungus (SF), non-sterilized control soil (NC), and non-sterilized soil with fungus (NF). The study aimed to analyze worm recovery, fecal eggs counts, and *A. galli* Igy levels after fungal administration. A significant interaction
between soil sterility and fungal treatment on ascarid worm burden was observed, which decreased significantly only in hens fed with sterilized soil inoculated with *P. chlamydosporia*, in comparison with the other three treatments. However, this scenario was completely different from that observed for egg counting, in which the overall EPG in the SF group was significantly higher than in groups SC and NC, but not versus the NF group. In addition, hens from the SF group had significant higher proportions of the three largest worm length categories (1.5–3.0 cm, 3.0–5.0 cm, 5.0–8.0 cm), in comparison with the other groups. This was an interesting result since the SF group had the lowest mean worm burden of *Ascaridia galli* and the highest abundance of mature worms, which allowed to conclude that reduced exposure modified *A. galli* populations. As stated by the authors, if all ascarid forms are not eradicated from the farm’s soil or litter, the remaining eggs might therefore lead to long-term serious infection outbreaks in flocks. These results emphasize the need to optimize parasite control programs in farms, targeting the reduction of environmental contamination with eggs and thus avoiding episodes of re-infection.

4. Further Research

Although only five research articles related with the use of predatory fungi against GI parasites of birds have been published to date, overall results reveal their potential effectiveness against nematode eggs and larvae and suggest their possible use in parasite control programs for domestic and exotic birds.

Despite their promising utility, some questions remain to be addressed. One of them refers to the impact of fungal administration on bird intestinal microbiota and if it can have a potential probiotic effect, besides their activity on fecal and soil environment. Interactions between the intestinal microbiota diversity and the chicken’s productivity has been demonstrated by several authors, although depending on the type of sample used for 16S rDNA sequencing (e.g., small intestine, large intestine, feces), with generally a higher bacterial diversity being found in the intestine of chickens with greater feed conversion ratio [54]. A growing number of studies aiming to characterize the relationships between parasites and the gut microbiota in several animal hosts has also been observed. For example, Huang et al. [55] demonstrated that, in chickens, coccidiosis modulated the avian gut microbiota towards a lower bacterial diversity and relative abundances of *Lactobacillus* and *Faecalibacterium*, in contrast to higher abundances of *Clostridium*, *Lysinibacillus* and *Escherichia* after fecal analysis. Therefore, it would be interesting to analyse the influence of predacious fungi administration on host intestinal microbiota, and to investigate if they can have a potential dual action on parasitism by regulating the gut microbiota and predating environmental forms.

More in vitro studies are needed to test these fungi against other bird GI parasites. Promising results already obtained against ascarid eggs and nematode larvae also reveal that it would be interesting to check the efficacy of ovicidal fungi against coccidia oocysts, cestode, and trematode eggs, as well as larvicidal fungi against L3 larvae from other nematode species. In addition, more in vivo studies using fungal formulations need to be performed in several species of domestic and exotic birds, kept in farms, zoos, or private collections, and evaluate the long-term kinetics of egg/oocyst shedding in the environment.

Since these fungi are often found in agricultural soils and animal feces, there is a great opportunity for scientific centres working on this topic to isolate native fungal species with predatory capacity and establish mycological collections, and routinely test them against GI parasites, namely from birds, both in vitro and in vivo, setting up the basis for developing more biocontrol products with market application.

**Author Contributions:** Conceptualization, J.L. and C.A.; methodology, J.L. and L.M.d.C.; validation, M.O., A.P.-S. and L.M.d.C.; investigation, J.L. and C.A.; resources, J.L. and L.M.d.C.; data curation, J.L.; writing—original draft preparation, J.L. and C.A.; writing—review and editing, M.O., A.P.-S. and L.M.d.C.; supervision, M.O., A.P.-S. and L.M.d.C.; project administration, L.M.d.C.; funding acquisition, J.L. and L.M.d.C. All authors have read and agreed to the published version of the manuscript.
Funding: This research was supported by CIISA—Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal, Project UID/B/00276/2020 (funded by FCT). Additionally, João Lozano was awarded a PhD research fellowship 2020.09037.BD (funded by FCT).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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