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# Sustainable Control of Equine Nematodes: *A Literature Review about Control by Fungal Cultures*

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**Abstract**

Larsen, Nansen, Grönvold, Wolstrup and Henriksen (1997) have previously reviewed the prospects of control of equine nematodes by using fungal cultures. Since then more investigations have been implemented and actualized. Field trials and laboratory tests have been reported with new perspectives and increasing number of animals in trials. The aim of this new review of literature is to add new knowledge to those questions that remained open in the 1990s. In particular, on the perspective of veterinary practitioner and sustainable use of deworming practices it is important to know those directions which should be followed.

**Keywords:** equine nematodes, field trials, fungal cultures, laboratory tests, resistance, sustainable use

## 1. Introduction

Some researchers are inspired by biological parasite control among cattle, sheep and horses because of increased drug resistance of nematodes. The subject of biological parasite control has been studied in Europe, Australia, United States and Latin America, but especially in Europe in Denmark. In 1997 Larsen, Nansen, Grönvold, Wolstrup and Henriksen have reviewed the prospects of control of equine nematodes by using fungal cultures. A net-trapping nematode-destroying fungus, *Duddingtonia flagrans* seem to be what innovators need because it can survive passage through the gastrointestinal tract of cattle and horses by its spores. Their review of literature covers investigations of those researchers and some abroad with 20 articles and based on this evidence it has been concluded that significant reduction in the number of infective larvae in the fecal environment were observed. This should lead to new prevention strategies including the use of nematode-destroying fungi (Larsen et al., 1997) and reducing number of commercial anthelmintic drugs.

Review of literature was published in 1997. Since then the resistance of nematodes has increased (Lind & Christensson, 2009; Kaplan, 2004; Näreaho, Vainio, & Oksanen, 2011; Canever et al., 2013; Lester et al., 2013; Lyons & Tolliver, 2013; Matthews, 2014), and increased awareness of social responsibility around resistance issues have been emphasized among individual citizens and even among pharmaceutical companies. At the same time, research methods have developed and new investigations of alternative parasite control are published. Nowadays the traditional approach to control should be abandoned and sustainable parasite control can be maintained (Nielsen, 2012). The aim of current review is to upgrade and critically re-evaluate the base of scientific knowledge about the use of fungi in the control of equine nematodes. By bringing new investigations under intensive discussion it is possible to evaluate this promising trend and its future directions.

### *1.1 History, Discovery and Benefits of Nematodes-destroying Fungi*

Nematodes-destroying fungi have been isolated from soil in Illinois in 1975 (Monoson, Conway, & Nelson, 1975) and in England as early as 1954 (Duddington, 1954). Selection and isolation of microfungi for use in biological control of parasitic nematodes in animals has been patented in 1997 (patent number US5643568 A). This is due to a close relationship between fungi and nematodes. It was already argued in 1981 that the fungi can cause the death of nematodes by parasitism (Tzean & Estey, 1981). Fungi can form a ring around a nematode and crush a worm with pressure. George Barron has described these events in his website on fungi.

Larsen et al have chosen the term “nematodes-destroying fungi” instead of “nematophagous fungi” because of the nature of fungi as the main or as a supplement food source of free-living soil nematodes. The use of nematodes-destroying fungi as a parasite control is also based on this knowledge. *Duddingtonia flagrans*, one member of Deuteromycetes group, is one with greatest interest. It has one characteristic typical of endoparasitic fungi. After eating it produces spores, and after germinating it grows and engulfs the contents of the nematode (Larsen et al., 1997).

Also fungi strains *Meristacrum asterospermum* and *Zygnemomyces echinulatus* have been identified as potential sources of biological control (Saikawa, Oguchi, & Ruiz, 1997). In general, 17 species of nematophagous fungi have been recorded (Kumar, R.K. Singh, & K.P. Singh, 2011). Three of them are endoparasitic. But it seems that one is still more important today in the control of animal nematodes, when the comparison has been made between *Duddingtonia flagrans* and other potential candidates (Zarrin, Rahdar, Poormohamadi, & Matehkolaei-Rezaei, 2017). The culture of *Duddingtonia flagrans* can be used either as a laboratory strain with a reference code ATCC 38101, as an isolate of AC001, as a Brazilian isolate of CG 768 or IAH 1297 (BioWorma®). Sometimes there is not an only *D. flagrans* fungi isolates in effectiveness studies, but *Monacrosporium thaumasium* (Tavela et al., 2013) and *Mucor circinelloides* (Hernández et al., 2016; Hernández et al., 2018) are also included.

Alongside the discovery of nematode-destroying fungi the sustainable environmental perspective with the specificity of biological control can be fulfilled. In controlling animal nematodes nematode diversity in acroecosystems must be first regarded (Yeates & Bongers, 1999). Around the world there is an intention to avoid chemical residues in the soil as well as in animals, and destruction of soil and species diversity. Earthworm population has an important role in soil fertility (M. Grdisa, Grsic, & M.D. Grdisa, 2013) so there is no need to kill them during the nematode control process. Destruction of target – only a target nematode – serves the intended purpose. Grønvold, Wolstrup, Larsen, Nansen and Bjørn (2000) have found the absence of the short-term impact of the nematode-trapping fungus *Duddingtonia flagrans* on survival and growth of the earthworm *Aporrectodea longa* and Yeates, Waller and King (1997) have shown similar non-effects on soil nematodes.

Chemicals have also adverse and unknown effects on animals and their environment. Safe use of fungi cultures without observable adverse effects of animals has not been studied until recently. Horses consuming fungi spore pellets have had normal values of erythrocytes, hemoglobin, and hematocrit and no signs of sensitization (IgG response) or respiratory, digestive or reproductive disorders (Hernández et al., 2016). Further, Hernández et al. (2018) have shown that none of the horses of their study (n = 22) rejected the pellets enriched with fungal spores and a normal appetite, digestive activity, reproductive behavior and breathing were recorded. In addition, no signs of skin damage or disorder were found during their latest experiment.

## 2. Inspections of Study Designs

### *2.1 Efficiency of Biological Control: Different Study Designs*

Firstly, the efficiency of biological control has been studied in field by feeding experiments (Fernández et al., 1997; Fernández et al., 1999; Baudena, Chapman, Larsen, & Klei, 2000; Braga et al., 2009; Paz-Silva et al., 2011; Tavela et al., 2011; Almeida et al., 2012; Hernández et al., 2018). For these purposes horses have been placed in pastures naturally infected with parasitic larvae and fungus-treated and control group have been established. Finally, the egg count per gram of feces has been recorded and success has been reported.

Secondly, the efficiency has been investigated in laboratory by isolation of fungal organisms from their usual surroundings to test tubes (in vitro tests). Significant decreasing the number of infected larvae have been found together with treatments by laboratory strains *Duddingtonia flagrans*, *Monacrosporium thaumasium*, and *Arthrobotrys robusta* compared to control group with no treatment (J.M. Araujo, J.V.Araujo, Braga, Carvalho, 2010; Arias et al. 2013; Tavela et al., 2013). This efficiency lasts for years even after seven years preservation period (Braga et al., 2011).

Thirdly, in laboratory in vivo tests in living organisms have been adopted. The capacity of supporting the passage through equine gastrointestinal tract without loss of ability has been tested together with egg count and control group with no oral dose (Araujo et al., 2010: Braga et al., 2010, Tavela et al., 2013; Buzatti et al., 2015). Activity has been investigated against strongylidae in general, *Strongyloides westeri* and *Oxyuris equi* (Araujo et al., 2010; Braga et al., 2010; Tavela et al., 2013) or more generally, by using horses with natural cyathostomins infection (Buzatti et al., 2015).

### *2.2 Relationship between Biological Control and Anthelmintic Delivery in Study Designs*

Despite the study design of biological fungi-based control anthelmintic therapy was not totally excluded. In the study of Paz-Silva et al this information is missing, but in general, animals in trials were received an anthelmintic treatment with ivermectin, which reduced the eggs per gram (EPG) of feces count to zero for investigation purposes (Baudena et al., 2000; Braga et al., 2009; Araujo et al., 2010; Braga et al., 2010; Tavela et al., 2011; Almeida et al., 2012; Tavela et al., 2013; Hernández et al., 2018). This procedure was carried out before the experiment (14 days was reported in studies of Araujo et al, Braga et al and Tavela et al). Sometimes the horses were treated several times before the experiment; with a single dose of ivermectin and five doses of oxlbendazole, and they were retreated if eggs reappeared (Baudena et al., 2000). Only Fernández et al (1997 and 1999) and Arias et al (2013) published their results without the use of anthelmintics, but with the use of naturally infected horses.

It is found by Hernández et al (2016) that horses provided with pellets containing added fungal spores did not need deworming towards *P. equorum* for 15 months. Then, however, the mixture of spores of *Mucor circinelloides* and *D. flagrans* were given daily to horses previously dewormed with ivermectin. Significant reduction of EPG was also observed during six months period when deworming and weekly administration of fungi pellets were integrated (Braga et al., 2009).

## 3. Conclusions

### *3.1 Generalizability of the Results*

In general, the results can be generalized when the bias of selecting cases can be evaluated. Based on this literature it is difficult to make such an evaluation. Sample size calculations and an origin and selection of horses were not justified except in Fernandez et al's study from 1997 in which it was reported that the horses were purchased from private farms. Number of horses in different studies did not always vary significantly (1, 3, 10, 12, 16, 21, 22, 25, 32), but after the grouping from treated to not-treated there were few cases (1-2, 4, 5, 6, 7, 8) in some group. Thus, parametric statistical analysis should have been changed to nonparametric analysis (Kruskal-Wallis test in Fernández et al's study and in Buzatti et al's investigation, Mann-Whitney U test in Paz-Silva et al's study and both nonparametric tests in the study of Hernández et al., 2018). Nevertheless, common analysis of variance, ANOVA and Tukey's test of multiple comparisons were widely adopted (Baudena et al., 2000; Braga et al., 2009; Araujo et al., 2010: Tavela et al., 2011; Almeida et al., 2012; Tavela et al., 2013) together with parametric t-test (Ferdández et al., 1999) and $\chi^2$- test with an assumption of normal distribution (Paz-Silva et al., 2011).

In everyday reasoning it is possible to think that when several researchers draw similar conclusions by several studies the generalizability is achieved. In addition, the controlling effect of nematodes-destroying fungi has been demonstrated in other species, among pig, cow and small ruminants (Larsen, 1999; Healey et al., 2018). However, excluding the study of Healey et al., the effect has been demonstrated by same active researchers by same methods in same climate conditions. Climate in Brazil, in which most of these studies have been carried out and in subtropical environment of southern Louisiana (Baudena et al., 2000), and in Spain (Paz-Silva et al., 2011; Hernández et al., 2016, 2018) cannot tell the utilization potential of fungal cultures in climates outside these countries. Fernández et al. (1997) have reported that dry climate conditions affected the transmission of infective larvae resulting in low numbers of larvae on the herbage, and during the rainy periods a significant reduction in the number of larvae recovered around fungal containing pats.

Also the predatory activity of *D. flagrans* can vary under different environmental conditions. The growth of this organism is visible in temperatures between 20-30 °C, (Bogus, Czygier, Kedra,, & Samborski, 2005), but on the predatory activity perspective it is more effective in 21 ℃ than in 28 ℃ (Paraud, Pors, Chicard, & Chartier, 2006). In some effectiveness studies the temperature of 30 °C has been seen as optimal (Buske et al., 2013). In recent cattle pasture study in Argentina predatory activity of *D. flagrans* has been evident in summer, but it has not been manifested in winter (Bilotto et al., 2018).

### *3.2 Usefulness the Results: Practical Perspective*

On the perspective of practicing veterinarian several questions are arisen about the control of equine nematodes by using fungal cultures. They are concerned with a life cycle of nematodes and a timing of a treatment. Although it is known that nematodes are destroyed by a special fungus in nematodes' body it is also known that infective larvae $L_3$ have no nutritional needs and thus, are not achieved by fungal methods except maybe by a crushing ring around a nematode. It is still unclear, based on these research reports, what is a convenient dose and feeding period. Daily treatments are previously and more recently recommended (Fernandez et al., 1997, 1999; Healey et al., 2018), but the dose administered to the animals may be adjusted according to the isolate employed (Buzatti et al., 2015). On the other hand, a single dose (Tavela et al., 2013), twice a week administration (Hernández et al., 2018) and longer intervals (Buzatti et al., 2015) can also be effective alternatives to the daily treatment.

In practice it would be useful to know the efficiency of biological control covering animals of all ages. Among current studies information of the ages of experiment animals were not always provided. Investigations were mainly focused on a) average 12 months of age, b) two years of age or c) 2, 5-3, 5 years of age except one study with focus group from three to eighteen years and two studies focused on “adult” horses. It is true that young horses, yearlings and foals under six months are the most sensitive to parasite infections, and therefore, should be treated in a different way than the older horses. Gras, Usai and Stancampiano (2011) have shown that host age is only partially consistent with the development of acquired resistance towards strongyles. Thus, the practitioners want to know what the deworming program for older horses is when the principle of biological control is introduced.

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