



Control of *Toxocara canis* with Nematophagous Fungus: Perspective to Public Health

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(Received 10 Sep 2020; accepted 22 Sep 2020)

Dear Editor-in-Chief

The WHO mentioned that from 2003 to 2012, approximately 1 million people were infected with geohelminths. The prevalence is directly correlated to inadequate sanitation, impoverished conditions and limited access to public health systems (1). Among geohelminths, *Toxocara canis* is noted as the principal etiologic agent of visceral larval migrans (VLM) in humans. Chieffi and Müller (2) reported it as a widespread parasitic disease in humans and a worldwide public health problem. Under favorable environmental conditions the eggs become embryonated harboring the second larval stage (L₂). Biological environmental control with nematophagous fungi is feasible and scientifically proven. It is targeted against eggs or larvae of most helminths (3). Hiura et al tested the helminthophagic activity of the predatory nematophagous fungus *Duddingtonia flagrans* on *T. canis*'s L₂. The assay was performed in 2% agar-water culture showing larvae's destruction. *D. flagrans* (AC001) also produces ex-

tracellular enzymes, especially chitinases and proteases, which are important for infection of nematode parasites (4, 5).

Thus, the objective of this study was to investigate the control of *T. canis* L₂ with *D. flagrans* as a future perspective in public health, under laboratory conditions in Brazil in the year 2019.

The study was approved by the Animal Ethics Committee of Universidade Vila Velha under process number 306.

Nematophagous fungus (isolated AC001) was isolated and cultivated in 2% chitin-rich-culture. *T. canis*'s eggs were obtained by hysterectomy dissection of adult female specimens. These eggs were embryonated for 30 days and at the end of this period, the *T. canis*'s second stage (L₂) were extracted, conditioned within microtubes in 24 h and divided in two experimental groups: treatment group (G1; 500 *T. canis*'s L₂ + conidia/chlamydo spores of AC001) and control (G2; 500 *T. canis*'s L₂ + destilated water). At the end of



24 h, there was 63% less recovery of L₂ in G1 when compared to G2 ($P < 0, 05$) (Fig. 1). In the present study, the percentage of reduction of L₂

treated with AC001 (63%) was in accordance with the records of Hiura et al (3) (53.3%).

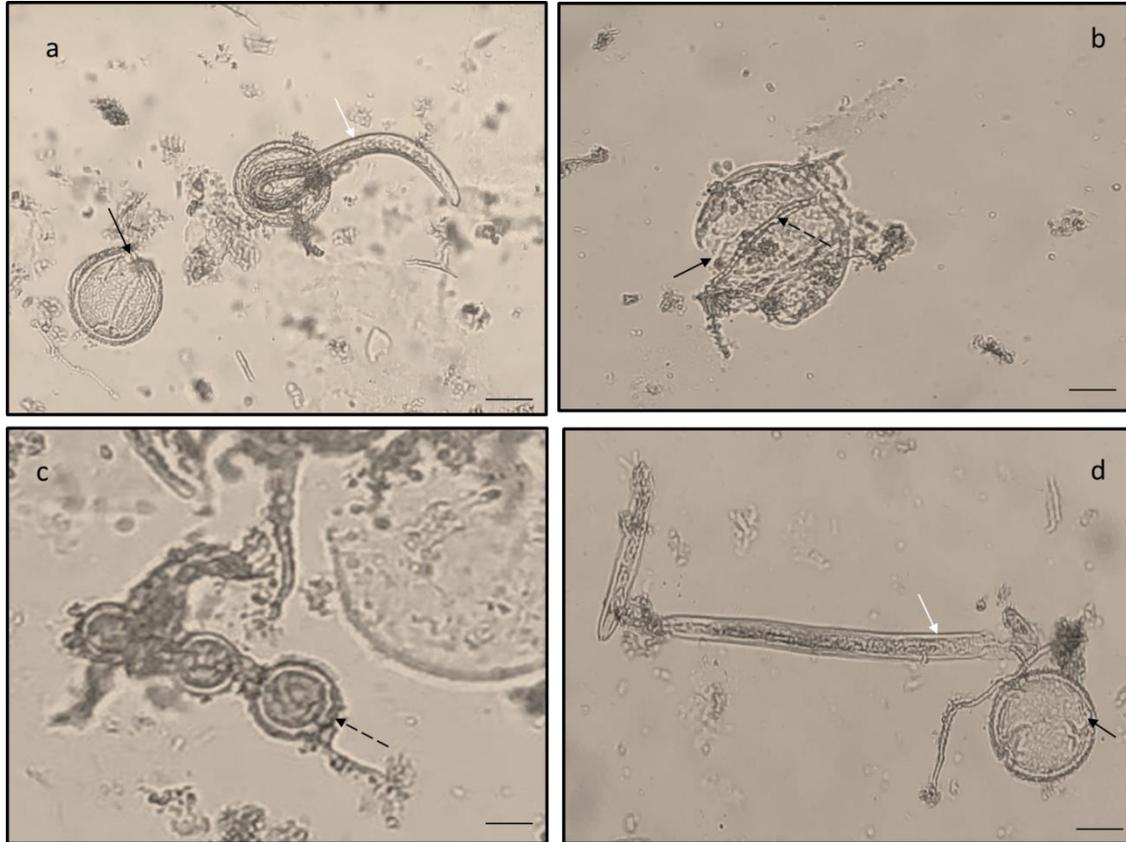


Fig. 1: a- Ruptured egg of *Toxocara canis* (black arrow) and second larval stage (L₂) of *T. canis* (white arrow). b- Ruptured egg of *T. canis* (black arrow) and hypha of *Duddingtonia flagrans* (dotted black arrow). c- chlamydospores of *D. flagrans* (dotted black arrow) and d – Disrupted second larval stage (L₂) of *T. canis* (white arrow) and ruptured egg of *T. canis* (black arrow). Light microscopy, 10x, 20 μ m scale

The Nematophagous fungi's expression of enzymes involved in the process of cuticle's degradation of nematode larvae was improved by the culture medium cultivating the *D. flagrans* in a medium rich in chitin (5). The cuticle of nematode is a complex extracellular structure composed mainly of proteins, with traces of lipids and carbohydrates (6). Thus, it is suggested that the interaction of *T. canis*'s L₂ and *D. flagrans*'s conidia/chlamydospores from chitin medium was enhanced due to the presence of chitinases and proteases in the extracellular medium, which interacted with the cuticle of *T. canis*'s larvae (5).

The results of the present study suggested that, in the future, fungal spraying of conidia/chlamydospores of *D. flagrans* (AC001) might be useful in the control of *T. canis*'s L₂ in contaminated environment (squares, parks and common recreation sites). In addition, the surface proteins of the conidia of *D. flagrans* should be the target of further studies.

Acknowledgements

To CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), CNPq (Conselho

Nacional de Desenvolvimento Científico e Tecnológico), FAPES (Fundação de Amparo à Pesquisa e Inovação do Espírito Santo) and Cooperação CAPES/FAPES (Programa de Desenvolvimento da Pós-Graduação – PDPG) for all the financial support throughout the group's research.

Conflict of interest

The authors declare that there is no conflict of interest.

References

1. WHO (2012). Soil-Transmitted Helminthiasis. Eliminating Soil-Transmitted Helminthiasis as a Public Health Problem in Children: Progress Report 2001–2010 and Strategic Plan 2011–2020. *World Health Organization*, Geneva, Switzerland.
2. Chieffi PP, Müller EE (1976). Prevalence of *Toxocara canis* in dogs, and the finding of ova of *Toxocara* species in the soil of public places in the urban areas of Londrina, State of Paraná, Brazil. *Rev Saúde Públ*, 10:367- 372.
3. Hiura E, Lopes ADCG, Paz JS, et al (2015). Fungi predatory activity on embryonated *Toxocara canis* eggs inoculated in domestic chickens (*Gallus gallus domesticus*) and destruction of second stage larvae. *Parasitol Res*, 114(9): 3301-3308.
4. Braga FR, Soares FEE, Araujo JM, et al (2014). Statistical experimental design to assess the influence of enzymes of nematophagous fungi versus helminths. *Res Vet Sci*, 97(3): 527-532.
5. Braga FR, Araujo JM, Araujo JV, et al (2013). In vitro predatory activity of conidia of fungal isolates of the *Duddingtonia flagrans* on *Angiostrongylus vasorum* first-stage larvae. *Rev Soc Bras Med Trop*, 46(1):108-110.
6. Fetterer RH, Rhoads ML, Junior JFU (1993). Synthesis of tyrosine derived cross-links in *Ascaris suum* cuticular proteins. *J Parasitol*, 79(2): 160-166.