Rev Iberoam Micol. 2017;34(1):32–35

Revista Iberoamericana de Micología

www.elsevier.es/reviberoammicol

Original article

Trichoderma virens as a biocontrol of *Toxocara canis*: *In vivo* evaluation

Fernando de Souza Maia Filho[a], Anelise Oliveira da Silva Fonseca[a], Beatriz Maroneze Persici[a], Julia de Souza Silveira[a], Caroline Quintana Braga[a], Luciana Pötter[b], Sônia de Avila Botton[b], Daniela Isabel Brayer Pereira[a,*]

[a] *Departamento de Microbiologia e Parasitologia, Instituto de Biologia, Universidade Federal de Pelotas, Pelotas, Rio Grande do Sul, Brazil*
[b] *Centro de Ciências Rurais, Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul, Brazil*

ARTICLE INFO

Article history:
Received 6 August 2015
Accepted 21 June 2016
Available online 18 January 2017

Keywords:
Biological control
Nematophagous fungi
Helminths
Experimental animal model

ABSTRACT

Background: Microorganisms have been widely studied as biological control agents of parasites of medical and veterinary importance. Coprophagous arthropods, bacteria and fungi are among the different organisms evaluated as potential biological control agents. Nematophagous fungi capture and digest the free forms of nematodes in the soil. Due to its zoonotic potential, *Toxocara canis* have been brought to the attention of researchers.
Aims: The aim of the present study was to determine whether the administration of embryonated *T. canis* eggs exposed to the nematophagous fungus *Trichoderma virens* reduces parasite infection in experimental animals.
Methods: Embryonated *T. canis* eggs were exposed to *T. virens* mycelium for 15 days at 25 °C. Subsequently, 100 fungus-exposed eggs were orally administered to 20 Swiss mice. As a positive control, another 20 mice received 100 embryonated eggs that were not exposed to the fungus. After 48 h, the animals were killed, and heart, lungs and liver were harvested for the recovery of larvae.
Results: The organs of the animals that received embryonated *T. canis* eggs exposed to the fungus showed a lower mean larval recovery when compared with the animals that received embryonated eggs without fungus exposure ($p < 0.05$).
Conclusions: The exposure of *T. canis* eggs to *T. virens* reduces the experimental infection, demonstrating the potential of this nematophagous fungus as a biocontrol agent.

Trichoderma virens como control biológico de *Toxocara canis*: evaluación *in vivo*

Palabras clave:
Control biológico
Hongos nematófagos
Helmintos
Modelo experimental animal

RESUMEN

Antecedentes: Algunos microorganismos han sido ampliamente estudiados como agentes de control biológico de parásitos de importancia médica y veterinaria. Los artrópodos coprófagos, las bacterias y los hongos están entre los diferentes organismos que sirven como agentes para el control con potencial biológico. Los hongos nematófagos capturan y digieren las formas libres de nematodos en el suelo. *Toxocara canis*, debido a su potencial zoonótico, ha captado la atención de los investigadores en estos estudios.
Objetivos: El objetivo del presente estudio fue evaluar si la exposición de huevos embrionados de *T. canis* al hongo nematófago *Trichoderma virens* reduce la infección parasítica en un modelo experimental animal.
Métodos: Los huevos embrionados de *T. canis* fueron expuestos al micelio de *T. virens* durante 15 días a 25 °C. Posteriormente, 100 huevos de *T. canis* expuestos al hongo fueron administrados por vía oral a un grupo de 20 ratones Swiss. Como control positivo se usó otro grupo de 20 ratones que recibieron 100 huevos embrionados no expuestos al hongo. Después de 48 h, los animales fueron sacrificados y corazón, pulmones e hígado fueron extraídos para la posterior obtención de larvas.

* Corresponding author.
E-mail address: danielabrayer@gmail.com (D.I. Brayer Pereira).

http://dx.doi.org/10.1016/j.riam.2016.06.004

Resultados: El número de larvas obtenidas en los diferentes órganos fue menor en el grupo de animales que fueron infectados con los huevos embrionados de *T. canis* expuestos al hongo en comparación con el grupo de animales que recibieron huevos embrionados sin la exposición al hongo ($p < 0{,}05$).
Conclusiones: La exposición de los huevos de *T. canis* a *T. virens* reduce la infección experimental, lo que demuestra el potencial de este hongo nematófago como agente para el control biológico.

Toxocariasis is a disease caused by the nematode *Toxocara canis* and different clinical forms have been observed, including visceral larva *migrans*, ocular larva *migrans* and occult or subclinical toxocariasis.[18] Infections in paratenic hosts primarily occur through the accidental ingestion of the embryonated eggs of the parasite, most frequently affecting children of up to five years of age due to increased contact with contaminated soil,[12] geophagy habit[21] and onychophagy.[2]

The use of microorganisms as biological agents acting on eggs and larvae of nematodes has been widely used in recent years as an alternative control method for nematodes. Thus, the nematophagous fungi are the microorganisms most studied for this purpose. These fungi live in the soil organic matter, develop parasitic or predatory relationships with nematodes, and are classified as ovicides, endoparasites and predators.[14]

Due to the problems caused by chemical control, mainly the prejudicial effects on human health and environment, the development of alternative control methods has become increasingly important.[21] Thus, biological control is a natural tool and an ecological alternative for the control of parasites of medical and veterinary importance. According to Araújo et al.,[4] biological control reduces infections caused by gastrointestinal helminth parasites, reflecting the use of living organisms as natural antagonists in the environment. Coprophagous arthropods, bacteria and fungi are among the different organisms evaluated as potential biological control agents. Nematophagous fungi capture and digest the free forms of nematodes in the soil.[20]

Among several genera of fungi evaluated for the biological control of gastrointestinal nematodes, *Pochonia chlamydosporia* and *Paecilomyces lilacinus* have exhibited ovicidal activity on *T. canis*.[6,11] Nevertheless, the genus *Trichoderma* has also shown ovicidal activity *in vitro* in *T. canis* eggs.[7,8,16] Additionally, this genus has been extensively studied and promising results, *in vitro* and *in vivo*, have been observed in the biological control of plant parasitic nematodes.[10,22]

The aim of the present study was to verify whether the exposition of embryonated *T. canis* eggs to the nematophagous fungus *T. virens* reduces the infection by this parasite in experimental animals.

Materials and methods

Fungal isolate

The fungal isolate used in the present study was obtained from the Mycology Laboratory of the Department of Microbiology and Parasitology at Universidade Federal de Pelotas (UFPel), Brazil. This fungus is an autochthonous isolate previously identified as *T. virens* based on morphological and molecular characteristics.

Obtention of T. canis *eggs*

T. canis eggs were obtained through hysterectomies performed on parasite females according to Maia Filho et al.[16] Subsequently, the eggs were maintained in a formalin solution (2%) containing streptomycin sulfate (0.05%) and chloramphenicol (0.01%). The eggs were embryonated after incubation at 25 °C/15 days with daily aeration.

Exposition of T. canis *eggs to* T. virens

One 4 mm-disk of fungal culture was transferred to Erlenmeyer flask containing 150 ml of modified minimal culture medium [NH_4NO_3 (0.4 g/l); $MgSO_4{\cdot}7H_2O$ (0.12 g/l); $Na_2HPO_4{\cdot}7H_2O$ (3.18 g/l), KH_2PO_4 (0.26 g/l), and yeast extract (0.3 g/l)]. A total of 5 flasks were inoculated with *T. virens* and incubated at 25 °C under gentle manual stirring twice a day during 15 days. On the 15th day, 500 embryonated *T. canis* eggs were added to each flask with the *T. virens* mycelium, and returned to incubation in the same conditions for further 15 days. Additionally, in the same day, 500 embryonated *T. canis* eggs were added to 5 flasks containing 150 ml of modified minimal culture medium, without *T. virens*, and were incubated in the same conditions previously described. Subsequently, the culture medium was centrifuged at 2000 rpm/5 min. The supernatant was discarded, and the pellet was suspended in 1 ml of 0.01 M phosphate buffer solution, pH 7.4 (PBS). To count and evaluate the eggs and viability, 10 μl of this solution were placed onto a slide, coverslipped and examined under a 40× objective. The eggs were considered viable when there was larvae inside, as described by Rey.[19]

Inoculation of experimental animals

Forty *Swiss* mice females (*Mus musculus*) of 4 weeks old were acquired from the animal facility at UFPel. The animals were maintained in appropriate cages at 25 °C, with water and food *ad libitum*. The animals were divided into two groups of 20 animals each: in group 1 (control) animals were infected by gavage feeding with 0.2 ml PBS containing 100 *T. canis* eggs, and in group 2 (fungus-exposed eggs) mice were infected by gavage feeding with 0.2 ml PBS containing 100 *T. canis* eggs exposed to *T. virens*. Forty-eight hours after the infection, the mice were killed by cervical dislocation. The liver, lungs and heart were harvested to recover the larvae. The organs were macerated and digested overnight in 50 ml of 1% hydrochloric acid solution and 1% pepsin at 37 °C with constant shaking at 120 rpm. Subsequently, the digested organs were centrifuged at 2000 rpm/5 min. The supernatant was discarded, and the total sediment of each organ was evaluated on glass slides using optical microscopy (10× and 40× lens) to count the larvae.[24]

All animal procedures were approved by the Ethics Committee on Animal Experimentation/UFPel.

Statistical analysis

The data for larval counting from the digested organs in both groups (group 1 and group 2) were submitted to a normality test using Shapiro–Wilk, Kolmogorov–Smirnov, Cramer–von Mises and Anderson–Darling tests. As the response variable did not show normality, data were subjected to the non-parametric chi-square test. In addition, data were submitted to analysis of measures of position