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Biological, biochemical and histological features of *Bradybaena similaris* (Gastropoda: Pulmonata) infected by *Heterorabditis indica* (Rhabditida: Heterorhabditidae) strain LPP1

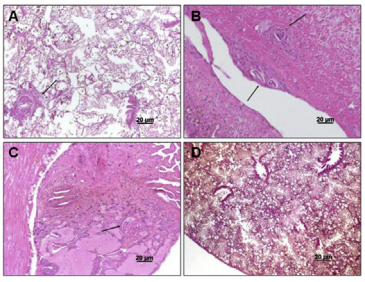
Victor Menezes Tunholi [a, *], Vinícius Menezes Tunholi-Alves [b, g], Caio Oliveira Monteiro [c], Lidiane Cristina da Silva [d], Cláudia de Melo Dolinski [e], Rosane Nora Castro [f], Vânia Rita Elias Pinheiro Bittencourt [g], Jairo Pinheiro da Silva [b, g], Isabella Vilhena Freire Martins [a]

[a] Departamento de Medicina Veterinária, Centro de Ciências Agrárias, Universidade Federal do Espírito Santo – UFES, Alegre, ES, Brazil
[b] Departamento de Ciências Fisiológicas, Instituto de Biologia, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, Brazil
[c] Programa de Pós-graduanção em Ciência Animal da Universidade Federal de Goiás – Avenida Esperança, s/n, Campus Samambaia, Goiânia GO, 74.690-900, Brazil
[d] Programa de Pós-graduação em Ciências Biológicas, Comportamento e Biologia Animal da Universidade Federal de Juiz de Fora, Rua José Lourenço Kelmer, s/n - Campus Universitário, Bairro São Pedro, Juiz de Fora MG, 36036-900, Brazil
[e] Universidade Estadual do Norte Fluminense Darcy Ribeiro, Centro de Ciências e Tecnologias Agropecuárias, Campos dos Goytacazes, RJ, Brazil
[f] Departamento de Química, Instituto de Ciências Exatas, Universidade Federal Rural do Rio de Janeiro, BR 465, Km 7, 23890-000, Seropédica RJ, Brazil
[g] Departamento de Parasitologia Animal, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, Brazil

HIGHLIGHTS

- *H. indica* LPP1 induce inversion in the oxidative metabolism of *B. similaris*.
- Exposure by *H. indica* LPP1 changes the levels of glucose and glycogen in *B. similaris*.
- *H. indica* LPP1 induces parasitic castration in *B. similaris*.
- *H. indica* LPP1 increase the activity of LDH in *B. similaris*.

GRAPHICAL ABSTRACT



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ABSTRACT

This study investigated the possible biological, biochemical and histological changes in *Bradybaena similaris* (Gastropoda: Pulmonata) infected by *Heterorhabditis indica* (Rhabditida: Heterorhabditidae), strain LPP1. Two groups of 16 snails were formed: the control group (unexposed) and the treated group, which was exposed for three weeks to infective juveniles (J_3) of *H. indica* LPP1. The experiment was conducted in duplicate, using a total of 64 snails. After the exposure period, the snails were dissected to collect the hemolymph and tissues, for evaluation of the physiological changes caused by the infection. The number of eggs laid/snail and the viability of these eggs were also assessed as indicators of the reproductive activity of *B. similaris*. Intense glycogenolysis was accompanied by a significant reduction ($p < 0.05$) in the glucose content of the hemolymph of the exposed snails, indicating that infection by *H. indica* induces breakdown of the host's glycemic homeostasis. Significant variations ($p < 0.05$) in the

* Corresponding author.
E-mail addresses: vinnytunholi@yahoo.com.br (V.M. Tunholi), vinicius_menezestunholi@yahoo.com.br (V.M. Tunholi-Alves).

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lactate dehydrogenase activity occurred together with changes in the concentration of pyruvic and lactic acid in the hemolymph of the infected *B. similaris* snails, corroborating the transition from aerobic to anaerobic metabolism in the hosts. These metabolic alterations reflect the parasitic castration process in this interface. The results suggest that the use of *H. indica* LPP1 is a potential alternative for biological control of *B. similaris*.

1. Introduction

The land snail *Bradybaena similaris* (Férussac, 1821) is a gastropod with great medical and veterinary importance, for acting as the intermediate host of trematodes and nematodes that affect the health of animals and humans (Alves et al., 2014; Tunholi-Alves et al., 2014). Among the relevant helminths, *Eurytrema coelomaticum* (Giard et Billet, 1892) Looss, 1907, a parasite of the pancreatic ducts of ruminants, stands out. It is endemic in various regions of the world, including South America, Asia and Europe (Bassani et al., 2006). The species is the etiological agent of bovine eurytrematosis, parasitic disease characterized by cachexia and anemia, resulting in significant economic losses from reduced production of meat and milk (Ilha et al., 2005; Quevedo et al., 2013). Additionally, epidemiological studies have demonstrated the participation of this gastropod as intermediate host of *Angiostrongylus cantonensis* (Chen, 1935), the main agent causing human eosinophilic meningoencephalitis. In recent decades that disease has spread to many regions of the world, and is currently classified as an emerging parasitosis (Wang et al., 2007; Morassutti et al., 2014). Due to the proven importance of this gastropod as a link in the transmission chain of these and other helminthiases, the World Health Organization (WHO, 1983) has urged control of the population of these organisms as one of the effective ways to eradicate these diseases.

The control of snails that act as intermediate hosts of parasites has traditionally been based on the use of chemical molluscicides (Machado, 1982). However, application of these compounds is not sustainable, showing low selectivity to the target organism and high ecotoxicity, posing a hazard to human and animal health (Henrioud, 2011). In reaction to this problem, experiments have been conducted to find new control alternatives, such as the use of plant-based molluscicidal substances and application of pathogenic microorganisms.

The use of molluscicides of plant origin is one of the most promising methods to control snails (Mello-Silva et al., 2010; Silva et al., 2012). In this respect, Lustrino et al. (2008) were the first to demonstrate the negative physiological effect of *Allamanda cathartica* I. (Apocynacea) on *B. similaris*. They observed a significant decline in the amount of galactogen stored in the albumen gland and of glycogen stored in the cephalopedious mass and digestive gland of the infected snails. Likewise, Mello-Silva et al. (2010), studying the molluscicidal action of the latex of *Euphorbia splendes* var. *hislopii* on *Biomphalaria glabrata* (Say, 1818), an intermediate host of *Schistosoma mansoni* (Sambon, 1822), observed alterations in the host's carbohydrate metabolism. According to the authors, the depletion of polysaccharide reserves resulted in relevant modifications in the snail's reproductive biology, specifically a reduction of the oviposition rate and egg viability.

Biological control agents such as fungi and pathogenic nematodes have also been indicated as promising alternatives to control snail populations (Jaworska, 1993; Rocha et al., 2009; Baron et al., 2013). In a recent study, Duarte et al. (2015) assessed under laboratory conditions the vulnerability of egg masses of *B. glabrata* to infection by *Metarhizium anisopliae*. The viability of the host's eggs and maturation of the egg masses diminished significantly after exposure to conidia and hyphal bodies of the fungus, suggesting the possibility of its use in programs for biological control of *B. glabrata*. Tunholi et al. (2014) for the first time described the pathogenicity of *Heterorhabditis indica* LPP1 in *B. similaris*. According to them, exposure to the nematode induced severe histopathological alterations in the host, resulting in a mortality rate of 55%. *Heterorhabditis* and *Steinernema* are genus of free-living nematode found in Brazilian soils widely, in which establish a symbiosis relationship with the bacteria of the genus *Photorhabdus* and *Xenorhabdus*, respectively (Burnel and Stock, 2000), being therefore used in insect control programs that act as agricultural pests (Hazir et al., 2003). The infection of nematode-infecting juveniles (J_3) in their natural hosts occurs through natural openings as (mouth, anus and spiracles) or, in some cases, through the cuticle. After entering the host's hemocoel, the nematodes release their simbiotic bacteria, which are primarily responsible for killing the host by septcemia (Dowds and Peters, 2002). In addition to the ability to infect a wide variety of arthropods (Dolinski et al., 2012; Monteiro and Prata, 2013), *H. indica* may accidentally infect gastropod snails, causing these hosts significants pathological changes (Jaworska, 1993). Li et al. (1986)has documented under laboratory conditions the susceptibility of *Oncomelania hupensis*, a semiaquatic snail that acts as an intermediate host of *Schistosoma japonicum*, to certain species of *Sterneinema* and *Heterorhabditis*. According to these authors, these nematodes had the ability to infect, develop and kill the host snail causing severe histopathological changes in the snail. Despite these studies, no information about the carbohydrate metabolism and reproductive parameters of *B. similaris* infected by *H. indica* LPP1 has yet been reported in the literature.

Therefore, to better understand the *B. similaris/H. indica* LPP1 relationship, the aim of this study was to evaluate the concentrations of glycogen in the digestive gland (DG) and cephalopedious mass (CPM), as well as the amounts of glucose and enzymatic activity of D- and L-lactate dehydrogenase (EC 1.1.1.27 and EC 1.1.1.28) (LDH) in the hemolymph of snails exposed to infective juveniles (J_3) of *H. indica*, strain LPP1, three weeks after exposure. In parallel, we measured the levels of galactogen stored in the albumen gland and performed histopathological analysis to shed more light on the reproductive alterations observed in this interface. We also applied HPLC to determine the levels of pyruvic and lactic acid in the hemolymph, to obtain conclusive information regarding the host's metabolic state.

2. Materials and methods

2.1. Source of the snails and nematodes

The snails used in this study were obtained from a colony kept in the Laboratório de Biologia de Moluscos do Museu Professor Maury Pinto de Oliveira of the Universidade Federal de Juiz de Fora (UFJF), located in the city of Juiz de Fora, Minas Gerais, Brazil. The nematodes of the species *H. indica* isolate LPP1 were donated by the Laboratório de Nematologia of Universidade Estadual Norte