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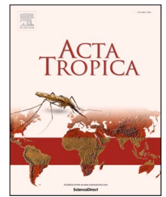

Heterorhabditis bacteriophora (Rhabditida: Heterorhabditidae), isolate HP88, induces reproductive and physiological alterations in *Biomphalaria glabrata* (Gastropoda: Planorbidae): an alternative for biological control of schistosomiasis

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ABSTRACT

Heterorhabditis bacteriophora is an entomopathogenic nematode (EPN) that is mutually associated with *Photorhabdus luminescens*, utilized globally for biological control of numerous organisms. Freshwater snails of the species *Biomphalaria glabrata* have been incriminated as the main intermediate hosts of *Schistosoma mansoni* in Brazil, but virtually nothing is known about the susceptibility of these gastropod to EPNs. Information in this respect is relevant for control of these intermediate hosts, and thus of the helminthiases they transmit. This paper for the first time reports the susceptibility of *B. glabrata* to infective juveniles of *H. bacteriophora* (isolate HP88) under laboratory conditions. For that purpose, six groups were formed: three Control groups (not exposed) and three Treated groups, in which the snails were exposed to 300 juveniles infecting the nematode over three weeks. The entire experiment was conducted in triplicate, using a total of 270 snails. Significant physiological alterations in *B. glabrata* were observed in response to the infection by *H. bacteriophora* HP88, characterized by decreased levels of hemolymphatic glucose as well as reduced contents of glycogen stored in the host's digestive gland. In parallel, the hemolymphatic activity of lactate dehydrogenase increased in the infected snails, indicating that the infection induces breakdown of carbohydrate homeostasis in *B. glabrata*. Additionally, all the reproductive parameters analyzed were reduced as a consequence of the infection. The results indicate the occurrence of the phenomenon of parasitic castration in the *B. glabrata/H. bacteriophora* HP88 interface, probably due to the depletion of galactogen in the parasitized organism. Although the infection did not cause lethality in the population of infected snails, *H. bacteriophora* HP88 compromised the reproductive performance of *B. glabrata,* suggesting its applicability in programs for biological control of this planorbid.

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1. Introduction

Schistosomiasis, caused by *Schistosoma mansoni*, is an infectious parasitic disease with widespread global distribution, and is endemic in various tropical regions as well as in countries with low socioeconomic development (Zoni et al., 2016). In Brazil, as in most other developing countries, the precariousness of public policies related to basic sanitation, sanitary education and access to health services contribute to the endemicity of schistosomiasis (Noya et al., 2015). Epidemiological studies carried out in Brazil revealed that the prevalence of schistosomiasis mansoni has significantly decreased in the last 40 years, reflecting a lower percentage of severe clinical forms (Katz, 2018). However, the disease is still a serious public health problem, being reported in various brazilian states with about 6 million people are thought to be infected (Gargioni et al., 2018). There are some 200 million people in the world who are infected by the parasite, and approximately 750 million live in areas of high risk of infection (Vos et al., 2012).

Various factors contribute to the endemicity of schistosomiasis, such as climate and the presence of gastropods that act as intermediate hosts, as well as of parasitized definitive hosts (Grimes et al., 2015). *Biomphalaria glabrata, Biomphalaria straminea* and *Biomphalaria tenagophilia* have been incriminated as the main intermediate hosts in Brazil, essential for dissemination of the parasitosis (Katz and Almeida, 2003). Due to these factors, the World Health Organization recommends not only treatment of the infected definitive hosts, but also development of methods for population control of the snails intermediate hosts.

For decades, the main method to control mollusks has been the use of chemical pesticides (Cantanhede et al., 2010). Among these base substances, niclosamide stands out for its high toxicity, with confirmed molluscicidal action (Machado, 1982). Despite this uncontestable molluscicidal efficacy, the use of chemical formulations in programs to control host snails has not been successful, due to the high cost, low selectivity and growing indications of the development of resistance to those compounds (Brasil, 2008). In response to this problem, the use of pathogenic microorganisms as biological control agents has been proven to be effective against various organisms, including ticks (Monteiro et al., 2020; Monteiro et al., 2014a; 2014c), insects (Leal et al., 2017) and mollusks (Duarte et al., 2015; Tunholi et al., 2017b). Wilson et al., (1995, 1994a) verified under experimental and field conditions, respectively, the ability of the nematode *Phasmarhabditis hermaphrodita* to infect, reproduce and kill different slugs. According to these authors, a directly proportional relationship was found between the dose of the nematode and the mortality of *Deroceras reticulatum*, confirming the potential of *P. hermaphrodita* as a biocontrol agent in gastropod snails.

The molluscicidal potential of *Heterorhabditis baujardi*, LPP7 against *Lymnaea columella* was demonstrated (Tunholi et al., 2017b). Besides causing high mortality, the exposure to the nematodes also induced relevant alterations in the reproductive parameters of the gastropod, in particular a decrease of the total number of eggs and reduced hatching rate. These results confirm the existence of the parasitic castration phenomenon, an important parameter for validation of biological control of the species. Despite that study and the results previously reported by Jaworska (1993) and Tunholi et al. (2014), validating the use of entomopathogenic nematodes (EPNs) for control of gastropod populations, no work has reported the susceptibility of *B. glabrata* to infection by EPNs.

Therefore, the objective of this study was to investigate the level of susceptibility of *B. glabrata* to infection by *Heterorhabditis bacteriophora* HP88, to better understand this relationship, with focus on the reproductive biology of the host and possible metabolic alterations undergone by this planorbid when infected to the nematode.

2. Material and methods

2.1. Source of Biomphalaria glabrata

The snails used in this study were obtained from a colony kept in the Laboratório de Biologia e Parasitologia de Mamíferos Silvestres e Reservatórios, Instituto Oswaldo Cruz (Fiocruz), located in the city of Rio de Janeiro, Brazil, thus confirming the age and sexual maturity of the specimens used as well as the absence of infections.

2.2. Source of the nematode Heterorhabditis bacteriophora, strain HP88

The nematode *H. bacteriophora*, strain HP88, were donated by the team of the Laboratório de Controle Microbiano, located at the Station for Parasitological Research W. O. Neitz of the Universidade Federal Rural do Rio de Janeiro (UFRRJ). For multiplication of the EPNs, caterpillars of the last instar *Galleria mellonella* (Pyralidae, Lepdoptera) were used, following the method proposed by Lindegren et al., (1993) and Kaya and Stock, (1997). The nematodes collected were stored in 40 mL cell culture bottles containing a solution of distilled water and placed in a climate-controlled chamber at 25 ± 1°C.

2.3. Experimental exposure to nematodes, formation of experimental groups and maintenance of snails

Sexually mature snails, with shell diameters of about 8–10 mm, were separated by groups into glass aquariums (12 × 24 × 14 cm) for experimental delineation. Infection was induced by exposure of the snails to an aqueous suspension adjusted to 300 infective juveniles (IJs)/snail, obtained from the cell culture bottles. Aliquots of 200 μL, containing the nematodes, were placed in the wells of 12-well plates and adding another 1800 μL of distilled water, then a randomly chosen snail was placed individually in each well. After exposure for 24 h, the snails were removed from the wells and transferred to aquariums. The wells were also examined with a stereoscopic microscope to check for the presence or absence of IJs of *H. bacteriophora* HP88.

The snails were separated into six groups of 15 each: the control group, formed by snails not infected to the nematodes, and the treated group, consisting of snails that had been infected to IJs of *H. bacteriophora* HP88. The entire experiment was conducted in triplicate, using a total of 270 snails. The snails were fed with lettuce leaves (*Lactuca sativa L.) ad libitum* and the aquariums were cleaned every other day, when the lettuce leaves were renewed to prevent their fermentation. The mortality was monitored daily until the end of three weeks after infection. The choice of this period (three weeks) was based on the results reported by Wilson et al., (1994b) regarding the mortality observed during infection of *Deroceras reticulatum* by *Heterorhabditis* sp.

2.4. Analysis of the reproductive biology of B. glabrata infected by H. bacteriophora HP88

The polystyrene plates were removed from the aquariums and the numbers of egg masses and eggs laid were counted under a stereoscopic microscope on alternate days until three weeks after infection. After the count, the plates were numbered individually and placed in new aquariums free of snails. Then the egg masses were observed to count the snails hatching. The egg viability, expressed as a percentage, is the number of snails hatched divided by the number of eggs laid in each experimental group, multiplied by 100 (Tunholi-Alves et al., 2011).

2.5. Dissection and collection of the hemolymph

Weekly and throughout the three weeks of study, the snails from the control and infected groups were dissected and the hemolymph was collected by cardiac puncture. Immediately after collection, Eppendorf® tubes containing the hemolymph were centrifuged for 10 minutes at

223g to remove the hemocytes and then stored at -10 °C until the biochemical analyses. All the samples were kept in an ice bath during dissection. Before proceeding with the biochemical analyses, the Eppendorf® tubes containing the hemolymph were centrifuged again for 10 minutes at 223g.

2.6. Determination of glucose concentration and LDH (lactate dehydrogenase) activity

An automated spectrophotometer Biosystems A15® was used for readings in these tests. The entire experiment was run in triplicate.

The protocol to determine the glucose concentration and enzyme activity followed the technical document from the manufacturer Biosystems® for each analysis. The protocol used was based on the method described, without modifications.

For the determination of glucose, a 10 μl of sample was added to 1 mL of color reagent (0.05 M phosphate buffer solution, pH 7.45 ± 0.1; 0.03 mM aminoantipyrine and 15 mM of sodium p-hydroxybenzoate; 12 kU of glucose oxidase, and 0.8 kU peroxidase per liter). The product formed by oxidation of 4-aminoantipyrine was determined by spectrophotometry with maximum absorption at 510 nm, using a standard solution of glucose at a concentration of 100 mg/dL. The readings were expressed in mg/dL.

For the determination of LDH activity, mixtures were prepared of 1 ml of solution containing substrate (0.1 M lactate solution, 0.005 M o-phenanthroline in 0.2 M Tris, pH 8.8), a drop of 0.012 M ferric ammonium sulfate and 25 μl of sample, and the mixture were incubated at 37 °C for 2 min. Next a drop of solution was added containing 15.82 mM of nicotinamide adenine dinucleotide (NAD) and 3.73 mM of phenazine metasulphate and the mixture was incubated at 37 °C for 5 min. The final reaction was stabilized by adding 1 ml of 0.5 M hydrochloric acid. After homogenization, the readings were taken in a spectrophotometer at 505 nm and the results were expressed in UI.

2.7. Determination of the glycogen and galactogen concentration

Glycogen contents stored in the gonad-digestive gland complex (DGG) and cephalopedal mass, and of galactogen stored in the albumen gland of snails infected and not infected to EPNs were determined according to the method 3.5 DNS and expressed as mg glucose/g tissue wet weight (Pinheiro and Gomes, 1994). The DNS (dinitrosalicylic acid) test is based on the reaction between reducing sugar and 3.5-dinitrosalicylic acid (yellow color), which is reduced to a reddish colored compound, 3-amino-5-nitrosalicylic acid, oxidizing the reducing monosaccharide.

2.8. Histological analysis

Three snails from each experimental group were dissected and transferred to Duboscq-Brasil fixative (Fernandes, 1949). Soft tissues were processed according to routine histological techniques (Humason, 1979). Paraffin-embedded sections (5 μm /LUPETEC® Model MRP2015) were stained using hematoxylin-eosin and observed under a Zeiss Axioplan light micro-scope. Images were captured by an MRc5 AxioCam digital camera and processed with the Axiovision software.

2.9. Statistical analysis

The data were first submitted to the Shapiro-Wilk test of normality, followed by analysis of variance one-way ANOVA and then the Tukey–Kramer test ($P < 0.001$) to compare the means (InStat, 200 GraphPad, v.4.00, Prism, GraphPad, v.3.02, Prism Inc.). The results were expressed as mean ± standard error for the evaluated reproductive parameters and as mean ± standard deviation for the analyzed biochemical parameters.

3. Results

Alterations in the reproductive activity of *B. glabrata* due to exposure to *H. bacteriophora* HP88 were observed during the three weeks of the study, differing significantly from the pattern of the control group. The exposition resulted in a significant reduction in the number of egg masses in relation to the unexposed snails (18.67±1.68), with the lowest values occurring in the third week of exposure (7.75±1.45, $P < 0.001$) (Fig. 1a).

The same trend was noted for the total number of eggs laid, with a reduction starting in the first week of analyses (209.3±15.9), representing a decrease of 56.36% in relation to the values of the control group (479.2±14.59). In the second week of exposure, the reduction was 54.52%, while in the third week the oviposition declined by around 58% (Fig. 1b).

With respect to the hatching rate, the exposure to *H. bacteriophora* HP88 also induced important variations, with significantly lower values than for the unexposed snails. In the first week of oviposition, the average hatching rate oscillated near 61.42% (128.02±23.52), representing a decline of 38.58% in relation to the total number of eggs laid (Fig. 2b). In contrast, in the control group, the hatching rate varied by nearly 95%, with mean values of (446.8±25.64) in the first, (423.3 ±10.05) in the second and (515.8±16.16) in the third weeks (Fig. 2a).

Exposure to *H. bacteriophora* HP88 also caused alterations in the oxidative metabolism of *B. glabrata*. In relation to glucose concentrations in the hemolymph, the average values observed in the infected groups were significantly lower than those in the unexposed groups, with decreases of 49.29% in the first week (35.75±5.9 g/dl), 56.22% in the second week (27.25±6.5) and 51.70% in the third week (28.5±2.65) (Fig. 3a).

An opposite variation was observed for the activity of lactate dehydrogenase in the hemolymph. In the first week, the exposition by *H. bacteriophora* HP88 induced a significant increase in enzymatic activity (16±0.81 IU), corresponding to a variation of 82% in comparison with the respective control group (8.75±1.6 IU). However, it was in the second week that the largest difference between the control and infected groups occurred (5±1.63 IU and 15.25±3.25 IU, respectively), representing an increase of 205% (Fig. 3b).

With relation to the levels of galactogen stored in the albumen gland of the snails, the exposition to *H. bacteriophora* HP88 caused a decrease of 77.87% (0.50±0.04 mg of glucose/g of tissue), differing from the value of the unexposed group (2.26±0.4 mg of glucose/g of tissue) after the first week of study (Fig. 4a). The same pattern was noted for the levels of glycogen in the DGG, with the smallest values being recorded in the second week of infection (3.8±1.03 mg of glucose/g of tissue) (Fig. 4b).

The histopathological analyses did not reveal significant alterations in the gonadal tissues of the infected snails compared to the unexposed ones. In both cases, the structure of the ovotestis appeared preserved, however, there was impairment of the gametogenesis step (Fig. 5). In addition, the results revealed the presence of larval stages of *H. bacteriophora* HP88 in the tissues of *B. glabrata*, corroborating the susceptibility of this planorbid to the nematode. However, consolidation of hemocytic inflammatory infiltrate surrounding the larval stages of the nematode was not observed (Fig. 6).

4. Discussion

Entomopathogenic nematodes have been extensively used as antagonistic agents for biological control of a wide range of organisms of medical and veterinary interest (Cardoso et al., 2013; San-Blas et al., 2019). Recently, studies have demonstrated the pathogenicity of nematodes (i.e. *H. indica* LPP1 and *H. baujardi* LPP7) for snails species, as *Bradybaena similaris* and *L. columella* (Tunholi et al., 2014; 2017a; 2017b), suggesting their use for control of important helminthiases. According to the authors, the infection by the EPN caused relevant

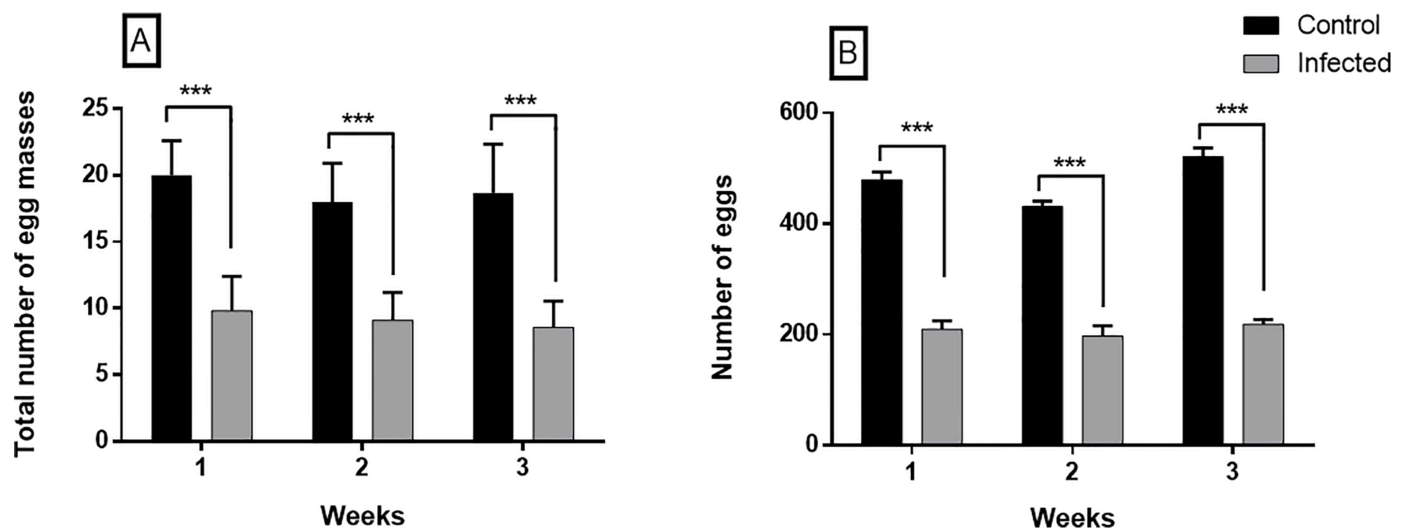

Fig. 1. Relationship established between the total number of egg masses (A) and the number of eggs (B) of *Biomphalaria glabrata* infected by *Heterorhabditis bacteriophora* during 3 weeks. (***) indicates that the means differ significantly between control and infected snail. p <0.0001.

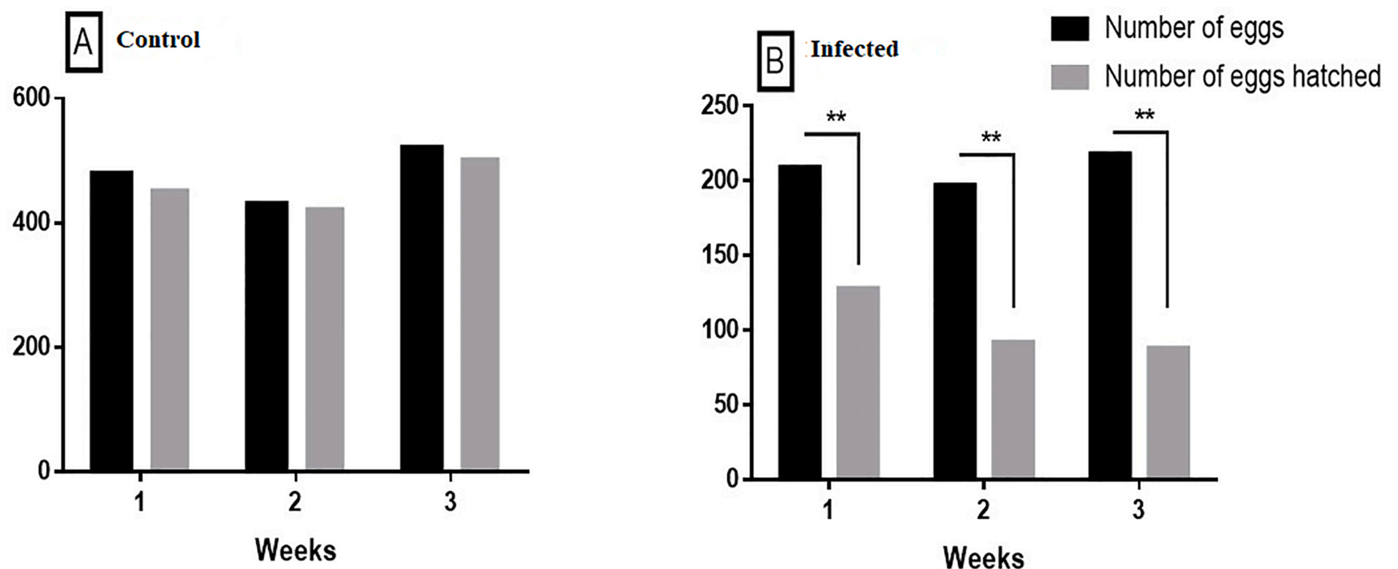

Fig. 2. Comparison between the total number of eggs and the number of eggs hatched between control snails (A) and experimentally infected to *Heterorhabditis bacteriophora* (B) during 3 weeks. (**) indicates that the means differ significantly between them. p <0.001.

metabolic alterations in the hosts snails, characterized mainly in the establishment of a negative energy balance; reproductive disorders such as reduced fertility, besides to course with significant mortality rates in infected snails' population.

Monteiro et al. (2013, 2014b, 2020) published a series of studies that validated, under experimental and semi-fields conditions, the use of *Heterorhabditis* spp. for the control of ixodid ticks. This was confirmed by the high mortality rate of the target population, as well as the reduction in the egg laying percentage of the females and the hatching rate of larvae. In parallel, an increase in the number of publications that confirm the susceptibility of gastropods to EPNs has been observed in recent years (Tunholi et al., 2014, 2017a, 2017b; Vidal et al., 2021). From this perspective, the present study reinforces the pathogenicity of EPNs for molluscs, providing the description of one more species that is susceptible to infection. Such studies have broken an old paradigm that believed that snails were resistant to infection by EPNs (Wilson et al., 1994). A similar condition was verified in ticks where Mauleon et al. (1993) stated that *Rhipicephalus microplus* was resistant to infection by EPNs, including *H. bacteriophora* HP88. However, it was later shown that EPNs are pathogenic for *R. microplus*, and that *H. bacteriophora* is one of the most virulent species.

Nematodes belonging to the genus *Heterorhabditis* are naturally isolated from soils (Ciche et al., 2006). During its life cycle, it checks the development of four juvenile stages (interspersed by molting), where the third stage (J3), considered the infective stage, demonstrates positive hydrotropism. The infection occurs actively by entry through natural orifices, and pathogenicity is attained by the action of bacteria released in the coelomic cavity of insects during the parasitic cycle (Han and Ehlers, 2000). In turn, the gastropod *B. glabrata* is an aquatic snail that is commonly found in stagnant pools and slow-moving watercourses in Neotropical regions (Teles, 2005). With both lungs and gills, adults and juveniles of *Biomphalaria* have amphibious behavior, with the ability to survive temporarily or even for long periods out of their natural aquatic habitat (Pieri, 1980). Thus, as a result of this behavior demonstrated by *Biomphalaria* spp. allows, under natural conditions, its exposure to *Heterorhabditis* spp.

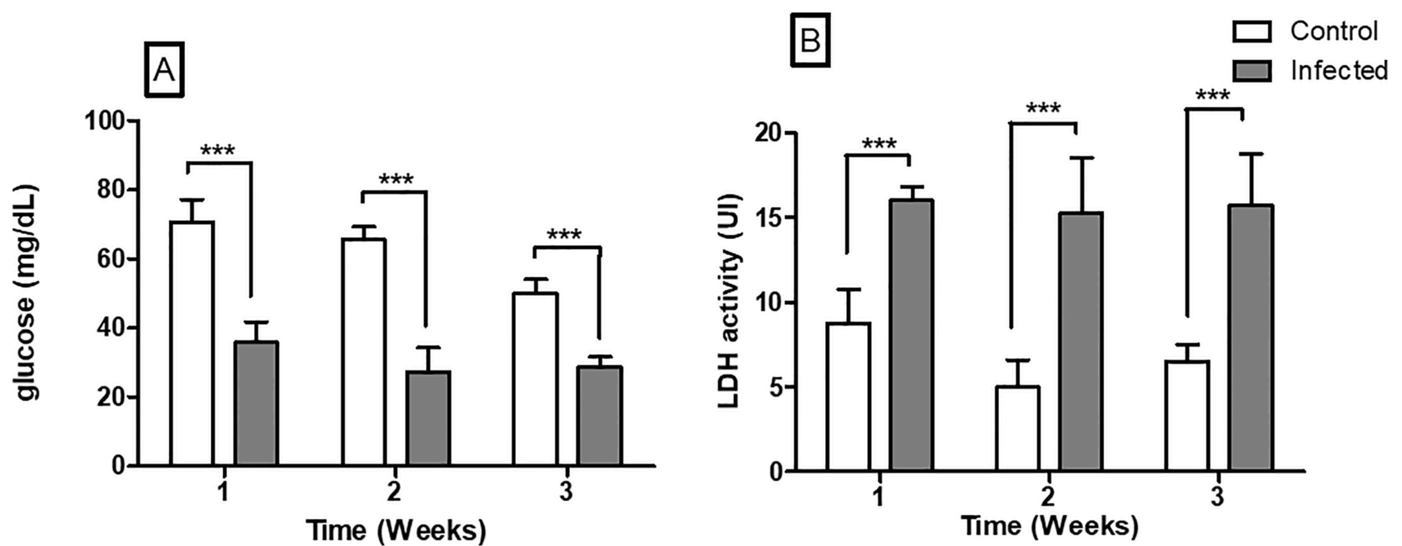

Fig. 3. Relationship established between glucose concentrations (A) and lactate dehydrogenase activity (B) in hemolymph of *Biomphalaria glabrata* experimentally infected by *Heterorhabditis bacteriophora*. (***) indicates that the means differ significantly between them. p <0.0001.

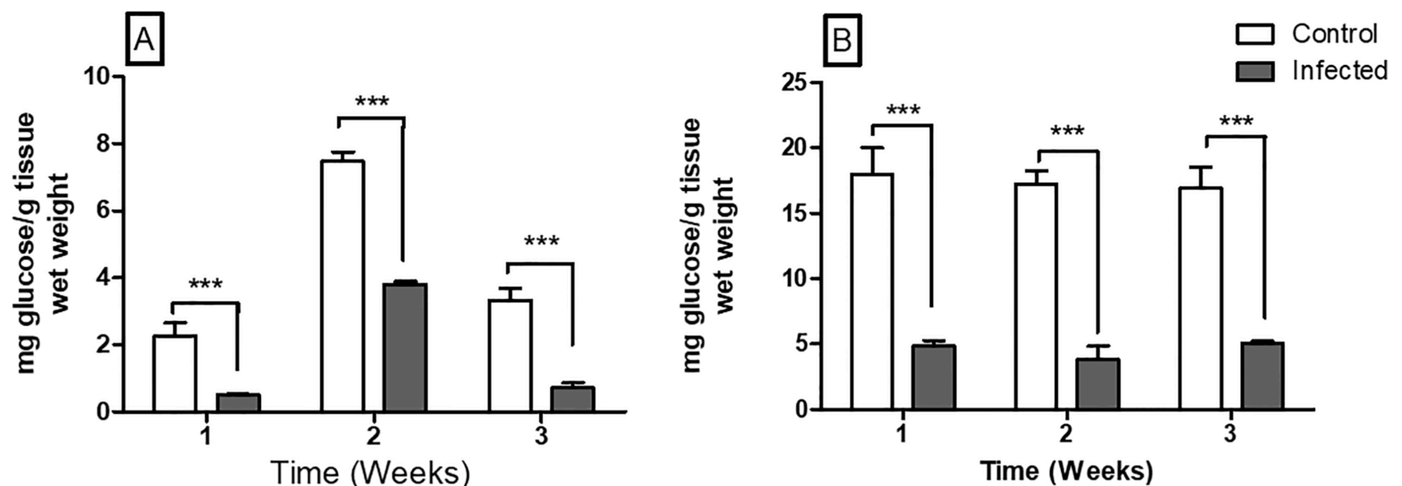

Fig. 4. Galactogen content in the albumen gland (A) and glycogen contente in the digestive gland (B), expressed in mg glucose/g tissue, fresh weight, in *Biomphalaria glabrata* experimentally infected by *Heterorhabditis bacteriophora*. (***) indicates that the means differ significantly between them. p <0.0001.

The experimental exposure of *B. glabrata* to IJs of *H. bacteriophora* HP88 resulted in significant alterations in the reproductive biology of the host, namely decreases in the number of eggs and egg masses laid, as well as reduction of the hatching rate of embryos. Various studies have demonstrated biological, histological and physiological alterations in mollusk/nematode interfaces (Bonfim et al., 2014; Lima et al., 2017; Martins et al., 2018). The parasitic castration phenomenon has been mentioned frequently in studies of the reproductive parameters of hosts (Lafferty and Kuris, 2009). According to Baudoin (1975), this process involves suppression total or partial of the host's reproductive parameters, associated with two possible causative mechanisms: (i) direct, when the host's gonadal structure is damaged by the mechanical action of the parasitic stages; and (ii) indirect, where the parasite causes depletion of the host's energy reserves. Based on this evidence, and to determine the physiopathogeny of the parasitic castration process, we performed complementary analyses to better understand it.

Significant reductions of the concentrations of galactogen in *B. glabrata* were observed during the three weeks of exposure to the nematode. Galactogen is a polysaccharide found only in cells of the albumen gland of gastropods, where it is considered to be the main constituent of the perivitelline fluid (Faro et al., 2013). This fluid is used by the embryos during the development stage for support of the intense metabolic processes and mitotic divisions. Therefore, in response to the depletion of the reserves of this polysaccharide, the fertility reduction phenomenon reported here results from indirect mechanisms, as a reaction to the weakened energy status of the host. A similar condition was reported by Tunholi et al. (2017a) studying the *L. columella/H. baujardi* LPP7 interface.

To better understand the effect of the parasitism on the host's energy status, we measured the concentration of glucose and the lactate dehydrogenase activity in the hemolymph, as well as levels of glycogen stored in the target tissues of *B. glabrata*. The exposure to *H. bacteriophora* HP88 induced a large reduction of the glucose concentrations compared to the unexposed snails. That physiological condition can partly be explained by competition for nutrients between the nematode/bacteria complex and snail. The ability of *Photorhabdus luminescens* (endosymbiont bacteria associated with this nematode) to utilize glucose as a carbon source was investigated by Jeffke et al. (2000). In turn, according to Gil et al. (2002), the growth rate of *H. bacteriophora* increased significantly when culture media were supplemented with glucose compared to the non-supplemented media. That result was attributed to the ability of *P. luminescens* to use glucose as an

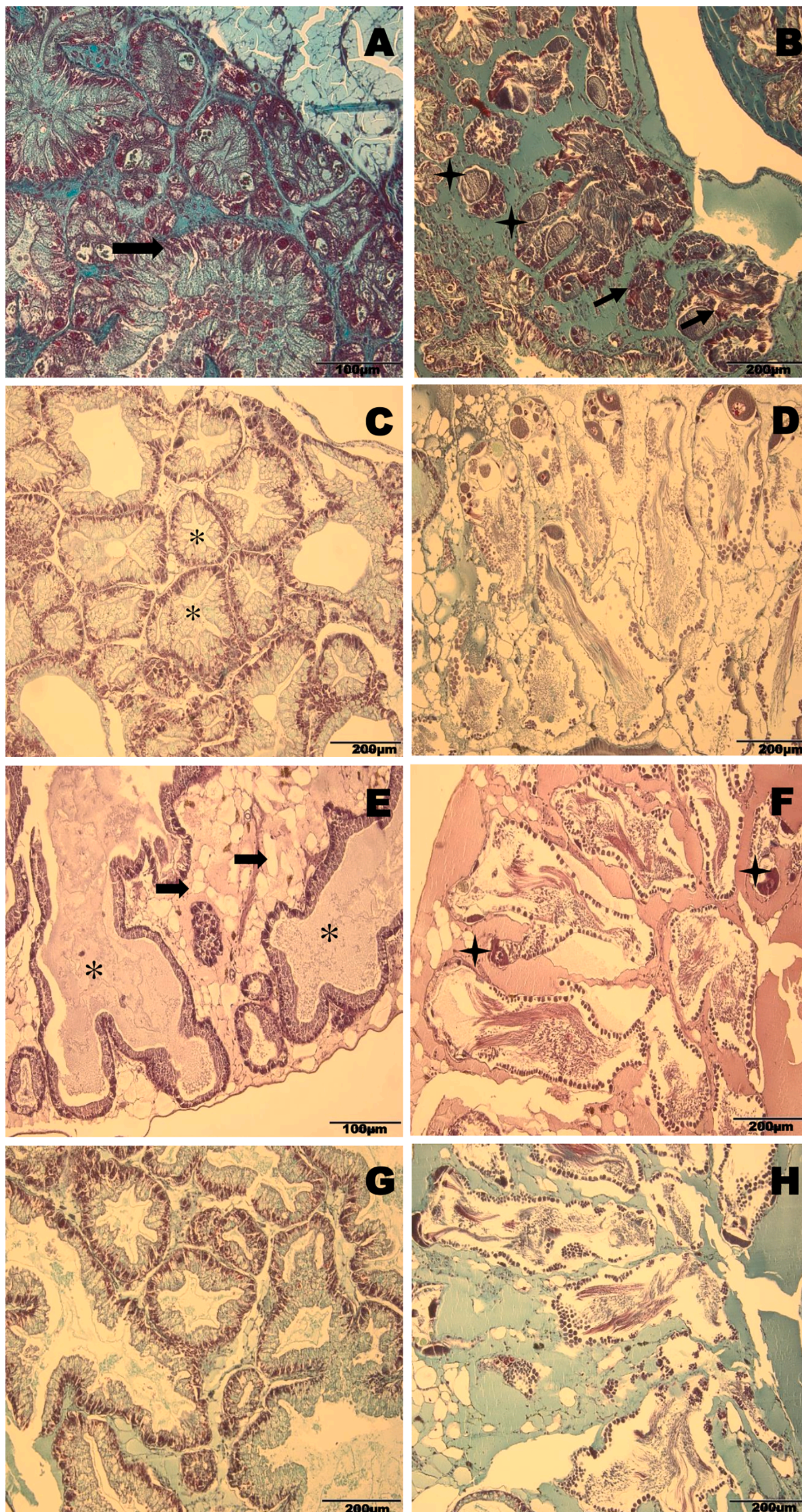

Fig. 5. Microscopic examination of the digestive gland (A, C, E, G) and ovotestis (B, D, F, H) of uninfected *B. glabrata*. Note the presence of a mucous cylindrical epithelium with nucleus in the basal position (➡) (A). Ovotestis with presence of spermatozoa (➡) and large numbers of oocytes (✦) (B). (C and D) *B. glabrata* infected with *H. bacteriophora* the first week. (C) cylindrical epithelium with small interacinar space (*) and (D) ovotestis with preserved oocytes and spermatozoa. (E and F) *B. glabrata* infected with *H. bacteriophora* second week. (E) Observe the loss of the cubic structure of the epithelium, increase in the interacinar space with accumulation of amorphous material (*) and areas of vacuolization (➡). (F) ovotestis with reduced and deformed oocytes (✦). (G and H) *B. glabrata* infected with *H. bacteriophora* third week. Same changes described in the previous group.

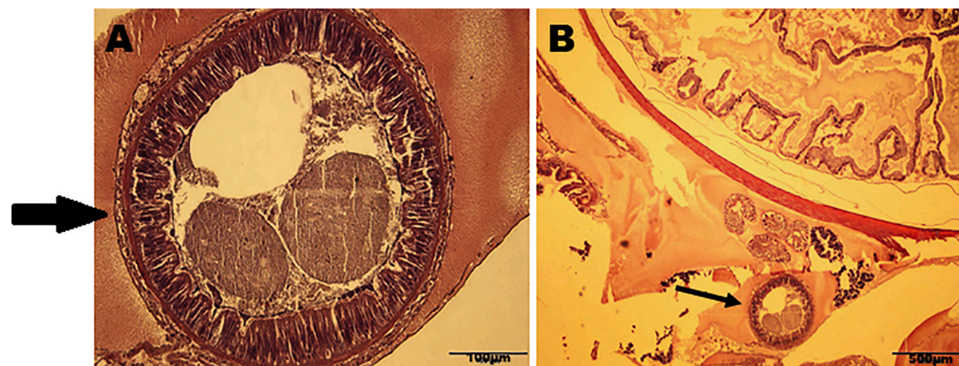

Fig. 6. Transverse section of *H. bacteriophora* HP88 situated after the palial chamber of *B. glabrata* (A and B), with two weeks of infection (➡)).

energy substrate, increasing the rate of bioconversion necessary for the nutrition of the nematode. Therefore, the hypoglycemia generated during exposure to *H. bacteriophora* HP88 compromises the formation of galactose, and consequently the synthesis of galactogen in the albumen gland of *B. glabrata*.

In response to the hypoglycemic state of the host, intense mobilization of glycogen in the DGG was verified, as an attempt to normalize the concentrations of that sugar in the hemolymph of infected snails. This fact elucidates the establishment of regulatory mechanisms, in particular between tissue and hemolymph (Mello-Silva et al., 2010). Other works have found that the depletion of energy reserves in target tissues, such as the DGG, results from the capacity for response of the host snail to adverse conditions caused by infection by helminths (Baudoin, 1975), prolonged fasting (Lira et al., 2000), and exposure to plant-based molluscicides (Silva et al., 2012). In our experiment, despite the decrease found in the glycogen levels, this was not effective in reestablishing the glycemic levels, suggesting that infection by this nematode induces breakdown of carbohydrate homeostasis in *B. glabrata*.

Increased intensity of fermentation reactions, such as those catalyzed by LDH, was observed in *B. glabrata* exposed to *H. bacteriophora* HP88. Similar condition was observed by Tunholi-Alves et al. (2018). Therefore, even though unfavorable to the snail from an energy standpoint, this condition is necessary to assure maintenance of its redox balance, guaranteeing via reoxidation of NADH and the use of new glucose molecules as a source of carbon to sustain important cellular pathways. In addition, Heermann and Fuchs (2008) confirmed, under experimental conditions, the endosymbiotic ability of *P. lusminencens* to establish itself in anaerobic environments, generating through fermentative oxidation of glucose ideal conditions for development of *H. bacteriophora.*

To validate the susceptibility of *B. glabrata* to the *H. bacteriophora* HP88 IJs, we conducted histopathological analyses Vidal et al. (2021)., studying the interface *Pseudosuccinea columella/H. baujardi* LPP7, verified at the end of the first and third weeks of exposure the presence of hemocytic infiltration in the digestive gland and mantle of the lymnaeid, respectively, resulting from the unleashing of an inflammatory process. The same authors also detected alterations in reproductive tissues of exposed molluscs, which are represented by the decline in the number of oogonias, oocytes and spermatogonias when compared to the control group. Similar results were observed by Tunholi et al. (2014). According to these researchers, the histochemical analysis revealed the presence of larval stages of *H. indica* LPP1 in the digestive gland of infected *B. similaris*, as well as the occurrence of granulomata with concentrations of haemocytes around the larvae of nematode. Unlike the response pattern observed by Vidal et al. (2021) and Tunholi et al. (2014), we did not find any similar manifestation. According to Ribeiro et al. (2003), the absence of hemocyte recruitment and formation of granulomas in insects results from the degradation of granulocytes by cytotoxins produced by the metabolism of bacteria. Hence, it appears clear that both the intensity and pattern of response vary widely in function of the interface studied (nematode species and strains/snail species), justifying the need for further research.

This work is the first report of the susceptibility of *B. glabrata* to infection by *H. bacteriophora*. Although the results did not demonstrate lethality in the population of infected mollusks, the process of fertility reduction and physiological alterations of the host were observed, suggesting the use of this bioagent for control of the planorbid. Our results are in agreement with those presented by Duarte et al. (2015) which validated the use of the fungus *Metarrhizium anisopliae* for population control of *B. glabrata* by impairing the viability and hatchability of its embryos. Based on the results obtained in the present study, it is believed that there is possibly an increase in the susceptibility of the snail to *Schistosoma* caused by the nematode infection. However, this hypothesis will be tested in future works using different co-infection dynamics.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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