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Changes in the locomotory and reproductive behavior of *Biomphalaria glabrata* infected with *Schistosoma mansoni*

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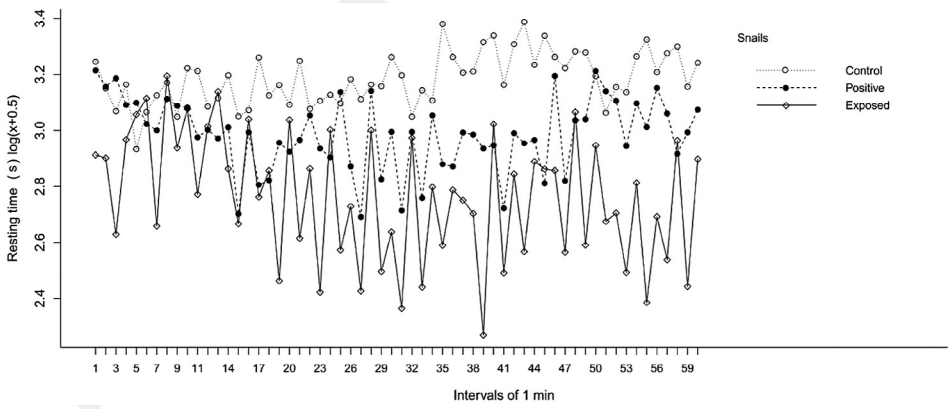
HIGHLIGHTS

- *B. glabrata* was monitored using an image analysis system.
- *S. mansoni* affects the locomotory and the reproductive behavior of *B. glabrata*.
- The number of cercariae shed is associated with the reduction in eggs-laying.

GRAPHICAL ABSTRACT



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ABSTRACT

The infection and development of a parasite may cause physiological, morphological and behavioral changes in its host. Changes in the locomotory activity of a host induced by their parasites may also influence the life-cycles of both host and parasite in the environment. The aim of the present work was to evaluate the locomotory activities of *Biomphalaria glabrata* before and after an experimental infection with *Schistosoma mansoni* relating to the shedding of cercaria. In addition, the reproductive parameters of infected *B. glabrata* were analyzed during the prepatent and patent periods of the infection. The locomotory activity was recorded using an image analysis biomonitoring system based on a Videomex V®. Five parameters were analyzed: distance traveled, ambulatory time, stereotypic time, resting time and average speed. The number of shed cercariae was counted twice at 45 and 52 days post-infection. The reproductive parameters of infected *B. glabrata* analyzed were the numbers of egg masses, eggs and hatched snails. All statistical analyses were performed using the R program. Of the 69 snails infected with *S. mansoni*, 33 (47.8%) shed cercariae ('positive') and 36 (52.2%) ('exposed') failed to exhibit any cercarial shedding prior to the end of the experiment. The locomotory activity of the all snails increased significantly after infection with *S. mansoni*. However, when the 'positive' and 'exposed' snails were compared, the former, shedding cercariae, were less motile. With regard to reproduction, 84.8% (28/33) of the 'positive' and 27.7% (10/36) of the 'exposed' snails failed to lay egg masses during patent period. The number of cercariae individually shed by each 'positive' snail

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presented a positive relation with stereotypic time and a negative relation with egg laying. Our findingshighlight the way in which infection with *S. mansoni* affects the locomotory and the reproductive behavior of *B. glabrata*. The number of cercariae shed is directly associated with the reduction/interruption in egg-laying and with an increase in random movement.

1. Introduction

Phenotypic, physiological, behavioral and morphological alterations have been described for *Biomphalaria glabrata* (Say 1818), which is in Brazil the main intermediate host of *Schistosoma mansoni* Sambon, 1907, in relation to different situations, such as parasitic infection, starvation, aestivation and exposure to (Faro et al., 2013; Mello-Silva et al., 2010, 2011).

Changes in the locomotory activity of *B. glabrata* infected with *S. mansoni* have been studied by several authors, including its movement in relation to various stimuli, such as light, depth and the use of molluscicides (Jurberg et al., 1987, 1988, 1995; Pieri and Jurberg, 1981; Sarquis et al., 1998). Boissier et al. (2003) reported that uninfected snails moved greater distances at faster rates and with shorter rest periods in comparison with infected snails. These authors also showed, in a specific experiment on attraction, that infected snails attracted other infected and uninfected snails, promoting aggregation, thus suggesting that this behavior could enhance the transmission of the parasite.

With regard to reproductive parameters, Faro et al. (2013) carried out a complete study of the process of parasitic castration in *B. glabrata* infected with *S. mansoni*. During the patent periods, the reproductive activity was regulated directly and indirectly by biochemical and histopathological variations caused by the developmental stage of the trematode. However, the parasitic castration was not correlated either to the parasite burden or to locomotory parameters. Experiments that associate reproductive and locomotory activity using the *Biomphalaria*/*Schistosoma* model have not yet been described. Therefore, it is important to elucidate the influence of the infection of *S. mansoni* on the behavior of the intermediate host and its possible effects on the transmission of parasite.

The aim of the present work was to evaluate the locomotory activity of *B. glabrata* using an image analysis biomonitoring system before and after an experimental infection with *S. mansoni* relating to the shedding of cercariae. In addition, the reproductive parameters of infected *B. glabrata* were analyzed during the prepatent and patent periods of the infection.

2. Material and methods

2.1. Ethics

This research was approved by the Animal Ethics Committee of the Oswaldo Cruz Foundation (CEUA-FIOCRUZ LW-19/13), in accordance with the guidelines of the Brazilian College for Animal Experiments (COBEA).

2.2. Experimental infections

Eighty specimens of *B. glabrata* (Belo Horizonte –BH lineage) born and reared in the laboratory, weighing 0.10–0.27 g, with a shell diameter of 8–12 mm and approximately 6 months old were used at the beginning of the experiments. The snails were fed *ad libitum* with fresh lettuce leaves (*Lactuca sativa* L.) three times per week, but were not fed for 12 hours prior to the video analyses. The snails were numbered and maintained individually in beakers of 10 ml with dechlorinated tap water and a controlled temperature (25 °C ± 2 °C) throughout the experiments. The water was replaced weekly. Each snail was exposed to 8–10 miracidia of *S. mansoni* (Belo Horizonte –BH lineage) obtained from experimental infection in "Swiss" mice aged 4–6 weeks according to the technique described by Fernandez et al. (2008). After 5 weeks, the snails were individually exposed to light for 1 hour in vials with 5 ml of dechlorinated water in order to check for cercariae. The cercarial number shed by each snail was counted in two screenings at 45 and 52 days post-infection using three aliquots of 0.5 ml from each vial. The cercariae were disposed on glass plates, fixed with Lugol's iodone and counted under a stereomicroscope. The total number of cercariae shed by each snail in each vial was estimated based on the mean number of cercariae counted from the three aliquots.

2.3. Locomotory activity

The image analysis biomonitoring system (IABS) was based on the use of a Videomex V® (Columbus Instruments, Ohio, USA) using the software Travelled Distance of Multiple Objects after Magalhães et al. (2007) and Santos et al. (2011). The instrument includes a recording cabin made of acrylate, diffused soft lighting and an analogical video camera. Inside it, an opaque glass aquarium of 30 l capacity (35 × 35 × 25 cm) contains four holding boxes (9.5 × 5.5 × 2 cm each) made of opaque acrylate with 3 mm holes, where the snail were placed individually during the experiments. Snails were kept in dechlorinated, filtered water with a controlled temperature (23.0 ± 1.0 °C) and pH (6.8 ± 1.0).

The biomonitoring of the snails took place twice: before the experimental infection with *S. mansoni* ('control group') and the same snails individually numbered were analyzed 40 days after the infection. The analyses of the locomotory activity of the same snails are essential to reduce the risk of the individual alterations, mainly related to behavior. Thus, we analyzed individually the same snails both before infection, with normal movement and without any stressor, and after the infection of *S. mansoni* during patent period. We elucidated that the snails had the same age, similar weight, nutritional status and the same infection pressure.

Infected snails that did not shed cercariae were called 'exposed', whereas snails shedding cercariae were designated as 'positive'. Each experiment was performed during a period of 1 h and 20 min, with 20 min of acclimation and 1 hour of video analysis recorded at 60 intervals of 1 min each. All values for each interval of five parameters of locomotory activity were used for statistical analysis: 'Distance traveled', 'Ambulatory time', 'Stereotypic time', 'Resting time' and 'Average speed'. 'Distance traveled' is the total distance (mm) traveled by the mollusc during the interval. 'Ambulatory time' is the total number of seconds during the interval which was spent in traveling movement. 'Stereotypic time' is the total number of seconds during the interval in which the mollusk performed some movement activity other than traveling, whereas 'Resting time' is the total number of seconds during the interval spent without movements. The 'Average speed' of animal movement was considered as the 'Distance traveled' divided by the 'Ambulatory time'.

2.4. Reproductive parameters

Reproductive parameters of infected *B. glabrata* such as fecundity (number of egg masses and number of eggs per snail) and fertility (hatched snail per eggs) were counted according to Mello-Silva et al. (2007) during the period of 9 weeks post-infection.

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