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**

**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 presented a positive relation with ‘Stereotypic time’ and a negative relation with egg laying. Our findings highlight 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.

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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 pre-patent 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 9 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 iodine 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 Videometrics 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 1 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 was 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 notshed 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.
2.5. Statistical analyses

All statistical analyses were performed using the R program (R Development Core Team, 2014). The analysis of variance (ANOVA) was used to determine differences between the weights of the snails groups (Control × Exposed × Positive). The weight data used for the ANOVA had a parametric assumption (Shapiro–Wilk: $W = 0.98$, $p = 0.15$). We used the generalized linear model (GLM) to estimate the association between the total number of cercariae shed per snail and the following variables: weight, mean value of each parameter of the locomotory activity, total number of egg masses, total number of eggs and total number of hatched snails. The chi-square test was used to investigate the number of eggs laid by the ‘positive’ and ‘exposed’ snails during the prepatent and patent periods of the infection.

We used the generalized estimating equation (GEE) to assess the locomotory activity of the snail before and after infection and to analyze the reproductive parameters of the ‘exposed’ and ‘positive’ snails. The model 1 was performed using the number of egg masses, eggs and hatched eggs per snail as a variable response. The GLM was used to calculate differences in the reproductive parameters of each specimen during each week of the infection.

To analyze the locomotory activity before and after exposure to miracidia, the model 2 was performed using each locomotory activity parameter as a variable response. Thus, we analyzed differences in the locomotory activity parameters of the ‘control’, ‘positive’ and ‘exposed’ snails and the interaction of weight on locomotory activity. Both models were adjusted to control over-dispersion. Finally, a goodness-of-fit statistic, the quasi-likelihood information criterion (QIC), was used for evaluating the models (Pan, 2001). The level of significance assumed for statistical tests was 5%.

3. Results

The first biomonitoring recording was performed with 80 uninfected snails that were subsequently exposed to miracidia of *N. mansonii*. Eleven snails (13.8%) died after the infection. Of the remaining 69 snails (86.2%), at 45 days post-infection, the cercarial shedding started and their weights were revaluated. Thirty-three snails (47.8%) were ‘positive’, weighed 0.18 (0.12–0.27) g and in two screenings at 45 and 52 days post-infection shed a mean number of 207 cercariae/snail. Thirty-six (52.2%) of the ‘exposed’ snails did not shed cercariae until the ninth week post-infection and weighed 0.17 (0.10–0.24) g. The primary weight of the 69 ‘control’ snails that subsequently survived the infection was 0.18 (0.11–0.27) g, and the ANOVA did not indicate any difference between the weights of all snails ($F = 1.03$, $p = 0.35$). Their weight did not influence the locomotory activity of the snails according to the GEE analysis.

With regard to reproduction, 84.8% (28/33) of the ‘positive’ snails failed to lay any egg masses during the patent period versus 27.7% (10/36) of the ‘exposed’ snails (chi-square test: $X^2 = 5.67$, $p < 0.05$). Of the ‘positive’ snails, the mean numbers of egg masses, eggs and hatched eggs per snail were 1.4, 10.5 and 4.2, respectively. Of the ‘exposed’ snails, the mean numbers for the same parameters were 1.9, 13.6 and 4.8, respectively. The GEE analysis showed a significant reduction of all of the reproductive parameters for each of the groups during the period of infection (Table 1).

When the ‘positive’ and ‘exposed’ snails were compared using a GEE analysis, the ‘positive’ snails significantly reduced the number of total egg masses in comparison with the ‘exposed’ (~28.8%) (estimate = -0.76, Wald = 18.57, $p < 0.05$). Although there were reductions in the numbers of eggs produced (~27%) (estimate = 0.10, Wald = 0.55, $p < 0.05$) and hatched snails (~20.1%) (estimate = -0.21, Wald = 0.45, $p < 0.05$), they were not significant. It is interesting that the weekly data show that ‘positive’ snails had an earlier decrease in egg masses and eggs (until the third week of infection), but in the hatched snails this was extended to the fourth week. After which, all the mean values became stable until the end of the experiment (Fig. 1A–C). Similarly, the ‘exposed’ snails exhibited a significant decrease in the number of egg masses, eggs produced and hatched snails, until the fourth week post-infection; subsequently, these parameters were stable.

The locomotory activity of each *B. glabrata* evaluated by GEE, both before and after the experimental infection, exhibited differences for all parameters. In general, all of the infected snails (‘exposed’ and ‘positive’) significantly increased their locomotory activity and decreased their ‘Resting time’ in comparison with the ‘controls’. The mean values for the parameters of the ‘control’ group were: ‘Distance traveled’ $2.9$ ($\pm 0.8$) mm, ‘Ambulatory time’ $4.3$ ($\pm 5.6$) s, ‘Stereotypic time’ $22.6$ ($\pm 14.9$) s, ‘Resting time’ $30.5$ ($\pm 18.2$) s and ‘Average speed’ $0.3$ ($\pm 0.5$) mm/s. The ‘exposed’ snails had mean values of ‘Distance traveled’ $3.9$ ($\pm 0.8$) mm, ‘Ambulatory time’ $6.1$ ($\pm 5.4$) s, ‘Stereotypic time’ $27.6$ ($\pm 12.6$) s, ‘Resting time’ $21.1$ ($\pm 13.1$) s and ‘Average speed’ $0.4$ ($\pm 0.4$) mm/s. The ‘positive’ snails had mean values for ‘Distance traveled’ $2.8$ ($\pm 0.5$) mm, ‘Ambulatory time’ $5.3$ ($\pm 4.3$) s, ‘Stereotypic time’ $28.5$ ($\pm 11.7$) s, ‘Resting time’ $23.5$ ($\pm 13.2$) s and ‘Average speed’ $0.4$ ($\pm 0.3$) mm/s.

The comparison between the ‘control’ and ‘exposed’ snails showed that the latter exhibited an increase in their ‘Distance traveled’ (estimate = -0.46, Wald: 4.06, $p < 0.05$), ‘Ambulatory time’ (estimate = 0.59, Wald: 4.24, $p < 0.05$), ‘Stereotypic time’ (estimate = 0.50, Wald: 3.15, $p < 0.05$) and ‘Average speed’ (estimate = 0.12, Wald: 3.66, $p < 0.05$), but a decrease in their ‘resting time’ (estimate = -0.42, Wald: 4.15, $p < 0.05$). Similarly, in the comparison between ‘control’ and ‘positive’ snails, the latter exhibited a significant increase in their ‘Distance traveled’ (estimate = 0.34, Wald: 3.04, $p < 0.05$), ‘Ambulatory time’ (estimate = 0.51, Wald: 3.69, $p < 0.05$), ‘Stereotypic time’ (estimate = 0.62, Wald: 4.39, $p < 0.05$) and ‘Average speed’ (estimate = 0.09, Wald: 3.17, $p < 0.05$), and a decrease in the ‘resting time’ (estimate = -0.19, Wald: 2.75, $p < 0.05$) (Fig. 2A–E).

No differences were found between the ‘positive’ and ‘exposed’ snails with regard to ‘Distance traveled’ (estimate = 0.12, Wald: 1.16, $p > 0.05$), ‘Ambulatory time’ (estimate = 0.07, Wald: 0.62, $p > 0.05$), ‘Stereotypic time’ (estimate: 0.12, Wald: 1.06, $p > 0.05$) and ‘Average speed’ (estimate: 0.02, Wald: 0.90, $p > 0.05$), with exception of ‘Resting time’ which was significantly higher for positive snails (estimate: 0.22, Wald: 2.33, $p < 0.05$) (Fig. 2A–E).

There was no significant difference in the number of cercariae individually shed by each ‘positive’ snail in relation to ‘Distance traveled’, ‘Ambulatory time’, ‘Average speed’ or ‘Resting time’. However, a positive relation was found for ‘Stereotypic time’ (estimate = 0.15, Wald = 2.75, $p = 0.01$) (Fig. 3).

We also assessed whether the number of shed cercariae was associated with the parameters of snail reproduction, but apart from the number of eggs, which showed a negative relationship (estimate = -0.01, Wald = 2.35, $p = 0.02$) (Fig. 4), there was no significant relationship with either egg masses or hatched snails (all $p > 0.05$).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>GEE model assessing the reproduction parameters of the positive and exposed snails regarding the number of egg masses, eggs and hatched snails during 9 weeks of the patent period</th>
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<tr>
<td>N. egg masses</td>
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</tr>
<tr>
<td>Exposed</td>
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<td>-0.38</td>
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<td>Hatched snail</td>
<td>Positive</td>
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<td>Exposed</td>
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4. Discussion

Parasites are known to have evolved the ability to manipulate host behavior in order to enhance their reproductive success (Moore, 2002). They can induce multidimensional phenomena, such as behavioral, morphological and physiological changes (Poulin, 2010, 2013; Poulin and Thomas, 2008). The present analysis, linking the reproductive parameters of *B. glabrata* with the locomotory activity under influence of infections with *S. mansoni*, gives new insights into this host–parasite relationship.

The image analysis biomonitoring system has been used to understand the behavior of different hosts of parasitic helminths. Santos et al. (2013) used the same methodology (Videomex V®) to study the locomotory activity of the freshwater snail *Melanoides tuberculatus* (Müller 1774), comparing a parasite-free group with others parasitized by the trematode *Centrocestus formosanus* (Nishigori 1924). These authors showed that in the infected snails, the movement was reduced and irregular, suggesting that less motile snails could possibly influence the life cycle of *C. formosanus* by concentrating the shedding of cercariae into a more restricted area. This

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**Fig. 1.** (A–C) Weekly analyses of the reproductive parameters of *Biomphalaria glabrata* experimentally infected by *Schistosoma mansoni*: (A) egg masses produced, (B) eggs produced and (C) hatched snails. Markers represent the points measured.
A biomonitoring system was also tested using fish infected with trematode metacercariae, which resulted in a significant decrease in the swimming behavior of the fishes correlated with parasite intensity, thus suggesting a possible disturbance in the predator–prey relationship in the natural environment (Santos and Santos, 2013). This image analysis biomonitoring system, based on a Videomex V®, is used for the first time in this study to evaluate the locomotory activity of B. glabrata, where it clearly demonstrated the effects of infections by the trematode *Schistosoma mansoni*.

Boissier et al. (2003) performed a comparative behavioral study of uninfected *B. glabrata* compared with others infected with *S. mansoni* using an aquarium with the bottom divided into

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**Fig. 2.** (A–E) Comparative graphics of the biomonitoring of *Biomphalaria glabrata* (control, exposed and positive) infected with *Schistosoma mansoni*, showing significant differences between 60 intervals of 1 min of monitoring in terms of (A) ‘Distance traveled’ (mm), (B) ‘Ambulatory time’ (s), (C) ‘Stereotypic time’ (s), (D) ‘Resting time’ (s) and (E) Average time (mm/s). Markers represent the points measured.

**Fig. 3.** Correlation graphic of the locomotory parameter of ‘Stereotypic time’. ‘Stereotypic time’ for positive *Biomphalaria glabrata* with the number of shed cercariae.
centimeters. The movements of individual snails were observed for 15 minutes and, based on visual observations, their Y-maze apparatus initially showed that infected snails moved less than uninfected ones. However, the locomotory and/or resting behavior of the snails could not be accurately measured and could have been influenced by the metabolic rate of each individual snail, a factor which was not taken into account. However, they observed an ‘aggregation behavior’ where both uninfected and infected snails were attracted by infected snails under laboratory and possibly in field conditions, as related by Sire et al. (1999). Following El-Ansary and Al-Daihan (2006), it seems that a reduction of the locomotory activity of Biomphalaria snails could be associated with a decrease in metabolism.

Changes in the metabolism of B. glabrata infected with S. mansoni have previously been reported by Becker (1980, 1983) in the form of a mobilization of the glycogen content from the digestive gland of the snail to the hemolymph mainly during the patent period of infection (period of shedding cercariae), and it also occurs in the infected snails exposed to the latex of Euphorbia milii (see Mello-Silva et al., 2010, 2011). These processes promote an acceleration of the metabolic which can increase the locomotory activity of the snail, such as that observed in our experiments.

In the present study a reduction in the reproductive parameters of ‘positive’ and ‘exposed’ B. glabrata were observed during the prepatent and patent periods, but in the ‘positive’ group they were more accentuated. That means that both groups were under stress caused by the infection, but this was more intense in the ‘positive’ due to their success in the shedding of cercariae. Hence, the greater number of cercariae shed is directly associated with a reduction or even total interruption of egg production. Faro et al. (2013) also reported an interruption in the egg-laying of B. glabrata during the patent period and showed that it was caused by a decrease in the energy resources and morphological alterations mainly in the ovotestis of the snails.

The comparison between the locomotory activity of infected snails (‘positive’ and ‘exposed’) and the control group, although measured in millimeters per second, showed an increase in displacement (‘Distance traveled’), during a longer period (‘Ambulatory time’) and at a greater speed (‘Average speed’), along with enhanced random movements (‘Stereotypic time’). Consequently, the infected snails decreased their ‘Resting time’. The subsequent comparison between the ‘positive’ and the ‘exposed’ snails showed there was no significant difference for the majority of the parameters in terms of their locomotory activity. However, the ‘Resting time’ was significantly greater in the ‘positive’ snails, thus leading us to infer that the pressure of the infection, together with the cercarial shedding, influenced the ‘positive’ snails to be less motile than the ‘exposed’ snails. With regard to the number of cercariae shed by the ‘positives’, there was a positive correlation with ‘Stereotypic time’, indicating a disturbed movement that does not represent a real displacement. One might suggest that cercarial shedding may cause some kind of discomfort. Our results, based on the biomonitoring analyses, showed that, contrary to other reports (Boissier et al., 2003; El-Ansary and Al-Daihan, 2006), infected snails moved more than the control group and snails shedding cercariae tend to be less motile than those that did not shed.

We conclude that the infection with S. mansoni affects the locomotory and the reproductive behavior of B. glabrata. Moreover, the number of cercariae shed is directly associated with the reduction/interruption in eggs-laying and with an increase in random movements.

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