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# INFLUENCE OF TEMPERATURE ON DEVELOPMENT OF *SCHISTOSOMA MANSONI* FEMALE CERCARIAE IN *BIOMPHALARIA GLABRATA.*

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## SUMMARY

In these experiments the ratio of male to female *S. mansoni* larvae in *B. glabrata* from Belo Horizonte and Ribeirão das Neves Minas Gerais, Brazil, either reared in laboratoty or collected in the field, varied from 1:1 to 1:1.3 or 1.4:1.

Cercariae of LE strain of *Schistosoma mansoni*, shed by 39 snails maintained at 25±0.5°C were used to infect mice on a weekly basis. Subsequent perfusion resulted in 76.6% male and 23.4% female worms. The cercariae produced by 32 infected snails maintained at 27±0.5°C were inoculated into mice and produced 43.4% male and 56.6% female worms ($p<0.05$).

Cercariae eliminated by snails collected in Barreiro and Ressaca, Belo Horizonte, during hot months, produced 45.7 to 47.7% male and 52.3 to 54.3% female worms. A lower number of cercariae shed by snails collected in Gorduras, Belo Horizonte, at 20±3.0°C, produced 51.6% male and 48.4% female worms. Thus, in this region the infection of vertebrate hosts with *S. mansoni* cercariae would be more severe in the summer due to the higher level of parasites and the number of eggs.

**KEYWORDS:** *Schistosoma mansoni*; Female cercariae; Development; Temperature; *Biomphalaria glabrata.*

## INTRODUCTION

MALDONADO & ACOSTA-MATIENZO[6] observed that the penetration of the host mollusc by *S. mansoni* miracidia could occur up to eight hours affter the hatching and was influenced by temperature. The authors verified that the reduction by 1.0°C in the maintenance temperature of the molluscs (from 26.0°C to 25.0°C), caused a reduction from 80-40% in the infection rate[2]. The evolution of the larva in the intramollusc phase to the hatching of the cercariae was under the influence of temperature and light[16].

STIREWALT[15], studying the effect of the maintenance temperature on the development of *S. mansoni* in *B. glabrata*, demonstrated that there was a variation in the duration of pre-patent periods, that were longer (35-56 days) at temperatures of 23.0 to 25.0°C and shorter (22-33 days) at temperatures of 26.0 to 28.0°C, with the shortest at 18 days at temperatures of 31-33°C. The best results were obtained in a range of 26.0 to 28.0°C, with an infection rate of 78% for molluscs individually exposed to five miracidia and 35% for molluscs exposed to one miracidium. At temperatures over 28.0°C the prepatent period was shorter, but the mortality was high.

This study was designed to detect factors that influence the invertebrate host-parasite interaction which is also relevant to the maintenance of the life cycle in the laboratory. The predominance of male worms in experimental infections of mice has already been observed by several authors[2,5,8,14]. Using the LE strain of *S. mansoni*, we generally observe an oscillation in the number of male and female worms obtained from bi and unisexual infections of mice. In this work we studied in detail the influence of temperature on the development of male and female larvae in the intramollusc phase in *B. glabrata.*

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Two mollusc maintenance temperatures have been tested during pre-patent and patent periods. To verify the ratio of male and female worms recovered from each experiment, mice were weekly inoculated with cercariae obtained from the two maintenance temperatures. Moreover, mice were inoculated with cercariae from molluscs captured in the field, during hot and cold months, and later observed for the ratio of male and female worms obtained using larvae from the two seasons.

## MATERIALS AND METHODS

*Biomphalaria glabrata* with 8-10mm of diameter which was derived from a strain which has been maintained in our laboratory for more than 20 years (provided to us in 1971 by the Interdepartmental Group of Studies on Schistosomiasis, GIDE, from Federal University of Minas Gerais) was used. Molluscs with natural infection were captured in the area of Ressaca, Belo Horizonte in March and September of 1988, May and June of 1989, and in Ribeirão das Neves, 48 kilometers from Belo Horizonte, in April and May of 1990, and April and March of 1991. Subsequently, we performed sampling with temperature registration of the water from breeding site in Barreiro de Baixo, Belo Horizonte, in April 1991 (temperature of approximately, 26.0°C) and in Ribeirão das Neves, in March 1992 (temperature of 25.0 to 29.0°C). Another group of molluscs with natural infection was captured in the region of Gorduras, Belo Horizonte, in June and July 1992, in water with the temperature of 17.5 to 23.0°C.

To infect the molluscs we used the LE strain of *S. mansoni* from Belo Horizonte, provided by GIDE in 1971 and since then maintained in this laboratory.

In the first experiment, 250 molluscs were exposed, in mass[13], to 50 miracidia and maintained in aquaria with approximately 152 ml of water per mollusc at room temperature (21.0 to 28.0°C). In the second experiment, 400 molluscs were individually exposed to one miracidium and maintained at room temperature under the same conditions of the first experiment. In the third experiment, 400 molluscs were individually exposed to 5 miracidia: 200 were maintained in an incubator (BOD) in plastic aquaria with 45 ml of water per mollusc at temperatures of 25.0 ± 0.5°C and the other 200 at 27.0 ± 0.5°C. The molluscs were fed with fresh lettuce and animal food. The water was changed and dead molluscs removed three times per week. After 30 days of exposure, molluscs were examined weekly until 50 days, selecting the infected individuals. Three mice were inoculated weekly with approximately 30 to 100 cercariae from each infected laboratory mollusc until all infected molluscs died. Molluscs from the field were exposed to light and the positive ones were selected. Following the selection of infected field molluscs, 30 to 100 cercariae were used for inoculation of three mice. Animals infected with cercariae from one mollusc were sacrificed and perfused after 50 to 55 days of infection. The number of adult male and female worms, as well as the number of molluscs with uni and bisexual infections were determined.

Swiss albino *(Mus musculus)* male mice with average age of 40 days obtained from the animal facility at Center of Research "René Rachou" were used.

Statistical analysis-numerical differences obtained from the different experiments were analyzed using the chi-square test, using the level of significance of 5% ($p \leq 0.05$), for all the calculations.

**TABLE 1**

Distribution of male and female *Schistosoma mansoni* larvae, LE strain, in laboratory *Biomphalaria glabrata* exposed to different numbers of miracidia and maintained at controlled or room temperature.

| Number of mollusc | Number of miracidia | Aquarium water temp. (°C) | Rate of infection(%) | Percentage of molluscs infected with larvae | | | |
|---|---|---|---|---|---|---|---|
| | | | | Male | Female | Bisexual | M:F |
| 131 | 1 | 21-28 | 34.7 | 49.6 | 50.4 | 0.0 | 1.0:1.0 |
| 39 | 5 | 25±0.5 | 82.5 | 28.2 | 5.1 | 66.7 | 1.3:1.0 |
| 32 | 5 | 27±0.5 | 70.7 | 15.6 | 31.2 | 53.1 | 1.0:1.2 |
| 50 | 50 | 21-28 | 94.9 | 0.0 | 2.0 | 98.0 | 1.0:1.0 |
| 102 | 71 | 21-28 | 95.4 | 1.0 | 3.9 | 95.1 | 1.0:1.0 |

M:F = Ratio of male to female larvae

## RESULTS

The ratio of male and female larvae, analyzed from 354 molluscs from laboratory, infected with 1, 5 or more *S. mansoni* miracidia was approximately 1:1 (Table 1). The proportion of male worms recovered from 2917 mice infected with the cercariae from those molluscs was significantly higher than the proportion of females, in the experiments at room temperature or at 25.0 ± 0.5°C (Table 2). On the other hand, in the experiments at 27.0 ± 0.5°C revealed a predominance of females (Table 2). The differences observed were statisti-

**TABLE 2**

Number of male and female *Schistosoma mansoni* worms, LE strain, recovered from mice inoculated with cercariae from *Biomphalaria glabrata* maintained at controlled or room temperature.

| Aquarium water temp.(°C) | Mean of cercariae | Worms recovered | | | | | Ratio M:F |
|---|---|---|---|---|---|---|---|
| | | Number | | Mean per mouse | Percentage | | |
| | | Male | Female | | Male | Female | |
| 21-28 | 100 | 6,169* | 4,576* | 30.6 | 57.4 | 42.6 | 1.3:1.0 |
| 25±0.5 | 94 | 22,544* | 6,904* | 32.7 | 76.6 | 23.4 | 3.2:1.0 |
| 27±0.5 | 86 | 12,348* | 16,125* | 24.1 | 43.4 | 56.6 | 1.0:1.3 |
| 21-28 | 100 | 6,78* | 4,216* | 34.7 | 62.0 | 38.0 | 1.6:1.0 |
| 21-28 | 100 | 5,022* | 4,196* | 31.6 | 54.5 | 45.5 | 1.2:1.0 |

*= Statiscally significant differences ($p<0.05$)
$x^2$ = 981.33, the cercariae from molluscs maintained at 21-28.0°C gave rise to a higher number of male worms.
$x^2$=121.60, the cercariae from molluscs maintained at 25 ± 0.5°C gave rise to a higher number of male worms.
$x^2$ = 25.35, the cercariae from molluscs maintained at 27 ± 0.5°C gave rise to a higher number of female worms.
$x^2$=95.54, the cercariae from molluscs maintained at 21-28.0°C gave rise to a higher number of male worms.
$x^2$ = 320.24, the cercariae from molluscs maintained at 21-28.0°C gave rise to a higher number of male worms.
M:F = Ratio of male to female worms

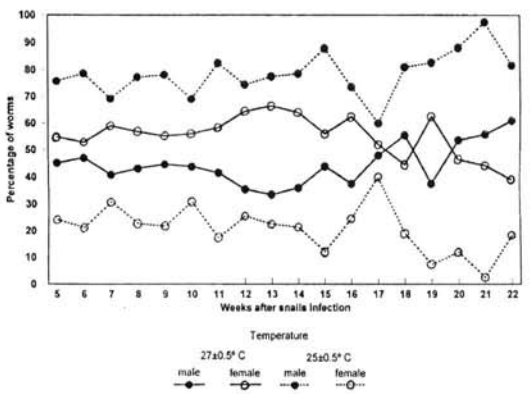


**Fig. 1** - Percentage of male and female worms recovered from mice inoculated weekly with cercariae from *B. glabrata*, each individually infected with 5 *S. mansoni*, LE strain, miracidia; 39 snails maintained at 25 ± 0.5°C, and 32 snails maintained at 27 ± 0.5°C.

cally significant ($p<0.05$) (Table 2). The proportion of male to female worms were 3.2:1 at 25.0 ± 0.5°C and 1.0:1.3 at 27.0 ± 0.5°C. The proportion of worms recovered in the experiments performed at room temperature varied from 1.2 to 1.6 males for each female.

Percentages of male and female worms recovered from the animals inoculated weekly with cercariae from molluscs maintained at 25.0 ± 0.5°C and 27.0 ± 0.5°C are shown in Figure 1. The mice were all perfused 50 to 55 days after infection with cercariae.

The mortality of 100% of the molluscs infected with 50 or 71 miracidium occurred 13 weeks (91 days) after infection. The median of mortality occurred at 36 days for the molluscs infected with 50 miracidia and at 50 days for the ones infected with 71 miracidia. The 100% mortality of the molluscs infected with 5 miracidia and maintained at 25.0 ± 0.5°C or 27.0±0.5°C occurred at 22 weeks (153 days) and 27 weeks (182 days) after infection, respectively. The median mortality in both experiments was 71 days after infection. From the seventeenth week after infection, the mortality of the molluscs reached 80% or more in both experiments decreasing the number of inoculated animals and changing the curves of recovery for male and female worms (Fig. 1). The longest life of the molluscs infected with male larvae was 35 weeks (245 days) and with females 22 weeks (154 days) after infection. The median mortality were 50 days for molluscs infected with male larvae and 57 days for the ones infected with females. The ratio of molluscs infected with male or female larvae was 1:1 (Table 1).

The ratio of male and female *S. mansoni* larvae found in 178 molluscs from the field was approximately 1:1 (Table 3). The ratio of male to female worms in 486 mice infected with cercariae from molluscs captured in three different areas around Belo Horizonte was approximately 1:1 (Table 4). There was a predominance of male worms using cercariae from molluscs captured in Gorduras during the cold months, and a predominance of females in the animals inoculated with cercariae from molluscs captured in Ressaca and Barreiro, in hot months. The differences were statistically significant ($p<0.05$). Infection of mice with cercariae from molluscs captured in Ribeirão das Neves gave rise to a ratio from 1.4 to 2.0 males for each female (Table 4). The differences were statistically significant ($p<0.05$).

## DISCUSSION

The influence of several factors over the biological cycle of *S. mansoni* in invertebrate and vertebrate hosts and over its sexual maturation has been well established in

**TABLE 3**

Distribution of male and female *Schistosoma mansoni* larvae found in *Biomphalaria glabrata* from the field captured at Ressaca (RE), Barreiro (BA), and Gorduras (GO), Belo Horizonte and Ribeirão das Neves (RN), during different periods from 1990 to 1992.

| Number of mollusc | Site of collection | Habitat water temp.(°C) | Rate of infection(%) | Percentage of molluscs infected with larvae | | | |
|---|---|---|---|---|---|---|---|
| | | | | Male | Female | Bisexual | M:F |
| 50 | RE | - | 7.3 | 36.0 | 34.0 | 30.0 | 1.0:1.0 |
| 19 | BA | 26 ± 1.0 | 8.0 | 36.8 | 52.6 | 10.5 | 1.0:1.3 |
| 32 | GO | 20 ± 3.0 | 6.8 | 25.0 | 40.6 | 34.3 | 1.0:1.2 |
| 52 | RN | - | 51.8 | 38.4 | 30.8 | 30.8 | 1.1:1.0 |
| 25 | RN | 27 ± 2.0 | 13.8 | 56.0 | 36.0 | 8.0 | 1.4:1.0 |

M:F=Ratio of male to female worms.

**TABLE 4**

Number of male and female *Schistosoma mansoni* worms recovered from mice inoculated with cercariae from *Biomphalaria glabrata*, captured in Ressaca (RE), Barreiro (BA), and Gorduras (GO), Belo Horizonte and Ribeirão das Neves (RN), during different .periods from 1990 to 1992.

| Site of collection | Habitat water temp.(°C) | Mean of cercariae | Worms recovered | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Number | | Mean per mouse | Percentage | | Ratio M:F |
| | | | Male | Female | | Male | Female | |
| RE | - | 100 | 2,352* | 2,792* | 38.1 | 45.7 | 54.3 | 1.0:1.2 |
| BA | 26 ± 0.5 | 96 | 765* | 850* | 27.7 | 47.4 | 52.6 | 1.0:1.1 |
| GO | 20 ± 3.0 | 62 | 816* | 765* | 15.9 | 51.6 | 48.4 | 1.0:1.0 |
| RN | - | 100 | 2,607* | 1,777* | 31.2 | 59.5 | 40.5 | 1.4:1.0 |
| RN | 27 ± 2.0 | 76 | 1,058* | 533* | 22.1 | 66.5 | 33.5 | 2.0:1.0 |

*=Statistically significant differences ($p<0.05$)
$x^2$=599.44, the cercariae from molluscs obtained from RE gave rise to a higher number of female worms.
$x^2$=80.0, the cercariae from molluscs obtained from BA gave rise to a higher number of female worms.
$x^2$=9.59, the cercariae from molluscs obtained from GO gave rise to a higher number of male worms.
$x^2$=23.25, the cercariae from molluscs obtained from RN gave rise to a higher number of male worms.
$x^2$=179.49, the cercariae from molluscs obtained from RN gave rise to a higher number of male worms.
M:F=Ratio of male to female worms.

the literature[1]. During the asexual phase in the host mollusc, several groups have observed that the normal ratio of sexual distribution of *S. mansoni* is 1:1[8,11]. However, in bisexual infections of animals with cercariae from those molluscs the ratio of male to female is 2:1[2,5,8,11,14].

According to EVANS & STIREWALT[3] and LIBERATOS[4] the higher number of male worms could be due to the low infectivity of the female miracidia for the molluscs. LIBERATOS[4] observed that the ratio of male to female worms in *Schistosoma* can vary from 1:5 to 5:0 and is related to the vertebrate hosts immunological response causing a selective destruction of eggs with female larvae[7].

In the Philippines, natural infection of *Rattus norvegicus* by *Schistosoma haematobium* leads to a ratio of male to female worms of 1:7. However, the proportion of male to female worms varied from 1:6 to 5:7 in mice from that region infected with *S. mansoni* [7].

In the present work, the relation of male and female *S. mansoni* larvae in laboratory as well as field derived *B. glabrata* was approximately 1:1[8,11].

The ratio of male to female worms varied from 1.2:1 to 1.6:1 in mice inoculated with cercariae from each laboratory mollusc maintained at room temperature. A ratio of 3.2:1 was found after maintaining the molluscs at 25.0 ± 0.5°C, which was higher than the ratio of 2:1 found by several other Brazilian researchers[2,5,8,11,14]. In temperatures of 27.0 ± 0.5°C the ratio was 1:1.3 with a predominance of females, but the proportion was approximately 1:1. Since the mice have the same origin and the molluscs and the LE strain of *S. mansoni* have been maintained in this laboratory for over 20 years, temperature differences are likely responsible for the discrepancy in these results.

These results agree with the observation of MALDONADO & ACOSTA-MATIENZO[6] and by STIREWALT[15] concerning the influence of temperature on the evolution of *S. mansoni* during the intra-molluscan phase and adds to the information about the evolution of the female larvae from LE strain in temperatures over 26.0°C. Laboratory molluscs infected with 50 and 71 miracidia had a shorter life span (91 days), as well as

bisexual infection rates over 95.0% which agrees with the observations of STIREWALT[14]. However, the rates of bisexual infections were under 70.0% for the individuals infected with 5 miracidia, but the life span of molluscs infected with 1 to 5 miracidia was longer than the life span of individuals infected with higher numbers of parasites. Molluscs with unisexual male infections survived up to 35 weeks (245 days), with female 26 weeks (182 days) and with bisexual infection 18 weeks (126 days). The life span of 35 weeks (245 days) was longer than that of 31 weeks (218 days) previously determined for *B. glabrata* from Belo Horizonte, with natural infection[12]. Of the mice infected with cercariae from molluscs captured in the field, we found a predominance of female worms in those infected with larvae from two regions of Belo Horizonte. However, the temperatures of water in the captures of these molluscs during the months of March, April, and September are unknown (Table 4). In the region were the capture happened in the cold months of June and July (17.5 to 23.0°C) we found a predominance of males. Additionally, the ratio of male to female worms obtained from animals infected with cercariae from molluscs captured in the three foci of Belo Horizonte was approximately 1:1. Cercariae from two captures performed during different periods of March, April and May, in the region of Ribeirão das Neves, gave rise to a higher number of male worms with a ratio of 1.4 to 2 males for each female, even in the hot months (25.0 to 29.0°C).

The rates of bisexual infection of molluscs from the field were lower than those from the laboratory animals. The rate varied from 8.0 to 34.3%, which is similar, in part, to the of 13.0% registered by PARAENSE & SANTOS[8] for *B. glabrata* from Belo Horizonte (Tables 1 and 3). The rates of natural infection of the molluscs from Belo Horizonte were under 10.0% (Table 3), which agrees with previous observations by SOUZA et al.[12]. The data herein show that in the field where several factors besides temperature can influence the mollusc-parasite interrelationship, such as: varying numbers of miracidia, different strains of *S. mansoni*, and immunological factors of the host[3,4,7,8,14], the seasonal variation of temperature less dramatically affect the development of *S. mansoni* female larvae, than in the controlled laboratory conditions.

Nowadays, among four of the foci analyzed here three have become extinct, Ressaca, Barreiro de Baixo and Ribeirão das Neves.

The results obtained using molluscs from the field indicated that in the municipality of Belo Horizonte, where the annual temperature of the water in the breeding sites can vary from approximately 17.0°C to more than 30.0°C, the transmission of schistosomiasis can be continuous since the molluscs survived and released infectious cercariae even in the coldest months of the year. However, the infection of vertebrate hosts in the hotter months could have higher numbers of parasites, with the predominance of female worms, and thus be more severe due to the production of a higher number of eggs.

## RESUMO

### Influência da temperatura sobre o desenvolvimento da cercária fêmea de *Schistosoma mansoni* em *Biomphalaria glabrata.*

Nestes experimentos a relação de larvas de *Schistosoma mansoni*, macho e fêmea, em *Biomphalaria glabrata* de Belo Horizonte e de Ribeirão das Neves, MG, Brasil, criada no laboratório ou capturada no campo variou de 1:1 a 1:1,3 ou 1,4:1.

Cercárias de 39 moluscos mantidos a 25 ± 0,5°C, inoculadas em camundongos originaram 76,6% de vermes machos e 23,4% de fêmeas; cercárias de 32 exemplares mantidos a 27 ± 0,5°C, originaram 43,4% de vermes machos e 56,6% de fêmeas ($p<0,05$).

Cercárias de moluscos capturados no Barreiro e Ressaca, Belo Horizonte, em meses quentes, originaram 45,7 a 47,7% de vermes machos e 52,3 a 54,3% de fêmeas. Moluscos capturados em meses frios (20 ± 3,0°C) no Gorduras, Belo Horizonte, produziram número menor de cercárias, que originaram 51,6% de machos e 48,4% de fêmeas. Portanto, nessa região, as infecções dos hospedeiros vertebrados com *S. mansoni*, poderão ser mais graves no verão devido a carga parasitária e número maior de ovos.

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