RESEARCH NOTE

# Partial Lack of Susceptibility to *Schistosoma mansoni* Infection of *Biomphalaria glabrata* Strains from Itanhomi (Minas Gerais, Brazil) after Fourteen Years of Laboratory Maintenance

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Several articles in the literatura have shown that some strains of *Biomphalaria glabrata* proved to be nonsusceptible when exposed to different samples of *Schistosoma mansoni*. A Godoy et al. (1997 *Mem Inst Oswaldo Cruz 91*: 121-122) reported successive generations of *B. glabrata that* were resistant to infection. Various authors have discussed factors possibly associated with this lack of susceptibility. According to CP Souza et al. (1995 *Mem Inst Oswaldo Cruz 92*: 317-322) resistance to the *S. mansoni* miracidium is partial, with no destruction of part of the sporocyst, which can evolve into a cercaria. CT Pan (1965 *Am J Trop Med Hyg 14*: 931-935) linked the resistance of *Biomphalaria* to the proliferation of amoebocytes and the formation of a protective capsule around developing larvae. According to WL Newton (1953 *Exp Parasitol 2*: 242-257), the developmental stage of the mollusk affects its susceptibility, which is greater among younger specimens. This correlation was also emphasized by LA Cooper et al. (1982 *J Parasitol 78*: 441-446) and MA Femandez (l997 *Mem Inst Oswaldo Cruz 92*: 27-29). The latter author showed a significant variation in the susceptibility of *B. glabrata* to infection between the first and third months of life.

Maintenance in the laboratory of *B. glabrata* strains from the rural area of Itanhomi, Rio Doce Valley, State of, Minas Gerais, since 1980 led us to the detection of a progressive partial lack of susceptibility to *S. mansoni* infection from autochthonous patients.The objective of the present article is to assess indices for susceptibility to *S. mansoni* infection in samples of young and adult *B. glabrata* from Minas Gerais from 1980 through 1994.

Eight groups of 30 *B. glabrata,* obtained from the rural area of the county of Itanhomi, were used. They were maintained in the laboratory of the Department of Tropical Medicine, Oswaldo Cruz lnstitute, Fiocruz, Rio de Janeiro, begining in 1980. Of the eight groups of mollusks, four consisted of young specimens which we exposed to 5, 10, 25, and 50 *S. mansoni* miracidia from patients with the hepatosplenic form of schistosomiasis who came from the same area where the mollusks were collected. The other four groups of mollusks were adults, exposed to the same respective numbers of miracidia. In addition, further eight groups, each with 30 *B. glabrata,* came from the same county in Minas Gerais, but were collected in 1994 and adapted in the laboratory. As in the previous stage of the experiment, four groups consisted of young mollusks and four were adults. Both groups were exposed to 5, 10, 25, and 50 *S. mansoni* miracidia. The experiment with mollusks gathered in 1980 and 1994 was developed at the same time and under similar conditions.

Temperature in the aquaria was kept between 24ºC and 26ºC. Fresh lettuce was used as the main food source for the mollusks, and the nutrient composition was based on B Rozemberg (1992 *Mem Inst Oswaldo Cruz 92*: 317-322). Infection of mollusks followed the technique recommended by G Chaia (l956 *Rev Bras Malariol D Trop 8*: 353-357). The method of WL Paraense and LR Corrêa technique (1989 *Mem Inst Oswaldo Cruz 84:* 281-288) was also used to determine elimination of cercariae. Mollusks were examined 30 days after having been placed in contact with *S. mansoni* miracidia. Specimens which did not test positive, were examined again at weekly intervals for up to 16 weeks. For comparative statistical analysis of infection indices in the different groups, we used the c2 test and contingency tables based on S

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Siegel (1956 *Nonparametric Statistics for the Behavioral Sciences*, McGraw-Hill, New York, 312 pp.), considering a significance level of 5% ($p<0.05$).

Among the young *B. glabrata* collected in streams in the county of Itanhomi in 1980, susceptibility to *S. mansoni* infection ranged from 75.3% to 81.4%, while in the group of adult mollusks the range was 68.3% to 85.5%. The young *B. glabrata* collected at the same location and adapted in the laboratory since 1980 presented susceptibility to *S. mansoni* infection that ranged from 7.7% to 16.3%. In the group of adult mollusks, the range was 3.7% to 4.9% (Table I). Among the younger strains of mollusks, kept in the laboratory since 1994, the positive index ranged from 51.5% to 57.1%. In the adult specimens the range was from 32.1% to 44.4% (Table II).

TABLE I

Susceptibility to *Schistosoma mansoni* infection in eight groups of 30 specimens of young and adult *Biomphalaria glabrata* collected in Itanhomi, Minas Gerais, in 1980

| | *B. glabrata* | | | | | |
|---|---|---|---|---|---|---|
| No. of | Young | | | Adult | | |
| miracidia | No. | Positive | % | No. | Positive | % |
| 5 | 26 | 3 | 11.5 | 27 | 1 | 3.7 |
| 10 | 25 | 4 | 16.3 | 23 | 1 | 4.9 |
| 25 | 28 | 3 | 10.7 | 24 | - | - |
| 50 | 26 | 2 | 7.7 | 25 | - | - |

No.: number of surviving snails.

TABLE II

Susceptibility to *Schistosoma mansoni* infection in eight groups of 30 specimens of young and adult *Biomphalaria glabrata* collected in Itanhomi, Minas Gerais, in 1994

| | *B. glabrata* | | | | | |
|---|---|---|---|---|---|---|
| No. of | Young | | | Adult | | |
| miracidia | No. | Positive | % | No. | Positive | % |
| 5 | 28 | 16 | 57.1 | 29 | 12 | 41.4 |
| 10 | 29 | 16 | 55.1 | 30 | 13 | 43.3 |
| 25 | 26 | 16 | 51.5 | 28 | 9 | 32.1 |
| 50 | 27 | 14 | 51.8 | 27 | 12 | 44.4 |

No.: number of surviving snails.

In the current study, susceptibility to *S. mansoni* infection for young *B. glabrata* kept under laboratory conditions since 1980 varied from 7.7% to 16.3%, while there was no direct correlation with an increase in the number of miracidia. Compared to susceptibility to infection in adult *B. glabrata*, where infection rates varied from 3.7% to 4.9%, there was a significant difference, with greater positive indices among young specimens. In terms of susceptibility among young mollusks collected from the same endemic area in 1994, the positive indices were higher than those obtained in adults for exposure to all the miracidial inoculates. Our results are in agreement with those of Newton (*loc. cit.),* who stressed the influence of the young stage of *B. glabrata* on the degree of susceptibility to infection. Studies by CS Richards and JW Merritt Jr (1972 *Am J Trop Med Hyg 21:* 425-434), and CS Richards (1984 *Malacologia 25*: 493-502) also showed this same correlation.

Beginning in 1980 MJ Conceição et al. (1993 Abst VI Int Symposium on Schistosomiasis p. 128) observed in the laboratory a gradual reduction in indices for susceptibility to infection in *B. glabrata* strains. When we compared infection rates in *B. glabrata* collected in 1980 with indices from mollusks gathered in 1994 we observed a significant difference, with higher positive indices in the group of mollusks recently adapted to the laboratory (1994). The majority of the mollusks kept in the laboratory since 1980 demonstrated partial lack of susceptibility to infection. This raises the question: What are the factors involved in this partial lack of susceptibility? Studies by CS Richards (1970 *Nature 277*: 806-810) already suggested a genetic basis in *B. glabrata* with relation to *S. mansoni* infection. Richards (1970 *loc. cit.)*, and CS Richards (1975 *Parasitology 70*: 231-241) correlated resistance in the adult mollusk to a single gene characteristic with a simple Mendelian pattern, with resistance being dominant. M Knight et al. (1991 *Exp Parasitol 73*: 285-294), THDA Vidigal et al. (1994 *Exp Parasitol* 79: 189-194) and THDA Vidigal et al. (1997 Abst VI Int Symposium on Schistosomiasis p. 43) have contributed with studies on genetic variations in *B. glabrata.* The latter used Amplified Polymorphic DNA analysis to differentiate between different Brazilian strains of the mollusk. One of the next stages of our study focuses on DNA characterization for *B.glabrata* which show a partial resistance to infection as compared to susceptible strains of the mollusk.

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