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STRAIN VARIATION IN THE INFECTIVITY OF SCHISTOSOMA MANSONI FOR BIOMPHALARIA GLABRATA(1)

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SUMMARY

Five strains of **Schistosoma mansoni** resistant and susceptible to schistosomicides were studied for infectivity of 2 strains of **Biomphalaria glabrata** one of Puerto Rican origin and the other of Brazilian origin.

Puerto Rican strains of **S. Mansoni** developed more slowly and had a lower infectivity in Brazilian **B. glabrata** than did the Brazilian **S. mansoni**. However, Brazilian **S. mansoni** developed as well in Puerto Rican snails as in Brazilian snails, indicating that drug resistant strains could easily be moved by travel of infected persons from one area to another.

KEY WORDS: Schistosoma mansoni; Biomphalaria glabrata; Strains, Experimental infectivity.

INTRODUCTION

It is well known that geographic strains of schistosomes vary in infectivity for their host snails[2, 6, 12-22]. This is of importance not only for the introduction of schistosomes into previously uninfected areas, but because of the potential for the spread of drug resistant strains of worms.

The present study was undertaken to determine if drug resistant strains of **S. mansoni** from Brazil and Puerto Rico were infective to **Biomphalaria glabrata** from the two countries. As these snails came from both the most northern (Puerto Rico) and the most southern (Brazil) points of their geographic ranges, the information would indicate how far the host parasite relationship has diverged.

MATERIALS AND METHODS

Snail Strains.

B. glabrata, an albino strain of Puerto Rican origin, has been maintained continuously in laboratory cultivation since 1953 (24 years at the University of Michigan and 10 years at the Center for Tropical Diseases, University of Lowell, U.S.A.).

B. glabrata an albino strain from Minas Gerais State, Brazil and of known susceptibility to **S. mansoni** has been maintained continuously in the laboratory for more than 20 years in the

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Strains of Parasites

BH (Belo Horizonte, Minas Gerais, Brazil).

This strain was isolated by **PARAENSE & CORRÊA**[16] from an untreated patient who contracted the infection at Belo Horizonte. Since 1967 we have been maintaining this strain continuously in the laboratory by passage through albino **B. glabrata** (Brazilian strain) and Swiss albino mice.

MAP (Minas Gerais, Brazil; oxamniquine and hycanthone resistant).

The MAP strain was obtained from a patient in 1978[7] following unsuccessful treatment with hycanthone and then oxamniquine. This strain has been maintained continuously in the laboratory by passage through albino **B. glabrata** (Brazilian strain) and Swiss albino mice.

H-30 (Johns Hopkins, Puerto Rico; hycanthone resistant).

This strain was derived by **in vivo** selection in mice treated with hycanthone in 1976[8] and has been maintained continuously in the laboratory by passage through **B. glabrata** (Puerto Rican strain) and CD_1 Swiss albino mice.

MPROXR (University of Massachusetts, Puerto Rico; oxamniquine resistant).

This strain was derived by **in vitro** selection of the MPR-1 strain[5] with oxamniquine and has been maintained continuously in the laboratory by passage through **B. glabrata** (Puerto Rican strain) and CD_1 Swiss albino mice since its selection in 1985.

MPROL (University of Massachusetts, Puerto Rico).

This strain was derived by **in vitro** selection of the MPR-1 strain[5] with oltipraz and has been maintained continuously in the laboratory by passage through **B. glabrata** (Puerto Rican strain) and CD_1 Swiss albino mice since its selection in 1985.

Each of these strains are currently maintained at the Center for Tropical Diseases, University of Lowell. The MAP and BH strains are also maintained at the Department of Parasitology, State University of Campinas.

The present study was carried out in the Center for Tropical Diseases, University of Lowell.

EXPERIMENTAL DESIGN

Breeding and Maintenance of Snails.

The procedures used for cultivation and maintenance of snails and parasite were adopted from **BRUCE & RADKE**[1] and **LIANG**[9].

Collecting Miracidia.

The method used for collecting miracidia was adopted from **LIANG, BOYD & BRUCE**[10]. Eggs were collected from feces of mice with patent infection of 60 days or more. A group of ten mice were allowed to defecate on saline damper paper towels placed on the bottom of the excreta pan of their cage. The feces were removed and mixed throughly with 100 ml of 0.85% saline. The suspension was poured into a tiered column of sieves arranged in descending order of mesh openings (420μ, 177μ, 105μ and 45μ). The eggs were washed through to the bottom sieve with 100 ml of 0.85% saline. A volume of 100 ml of aerated tap water was poured into the sieve column to rinse eggs free from saline. The eggs were washed from the bottom sieve into a petri dish (2 X 10 cm) with 40 ml of aerated tap water and concentrated to the center of the dish by gentle rotation. The eggs were then pipetted into a small dish (1.5 X 6 cm) and the dish was placed under ceiling illumination to induce hatching of miracidia. Miracidia usually appeared within 10 min. Snails were exposed to miracidia which were less than one hour old.

Exposure of Snails

Snails (3-4 mm in size) were recovered from petri dish cultures and were exposed individu-

ally. Snails were placed in glass vials (15 X 17 mm) containing 0.2 — 0.4 ml of aerated tap water. Each snail was exposed to 10 miracidia. The exposure temperature was 25-27°C and snails were left for 4 hours in the exposure containers under ceiling illumination. For each strain of **S. mansoni**, groups of 50 snails were exposed. Corresponding control groups (**B. glabrata** from Brazil and Puerto Rico) also consisted of 50 snails.

After exposure snails were maintained in groups of 25 per plastic tray containing 1.5 liters of aerated tap water and supplied with lettuce, blue-green algae, and mud. They were placed under continuous light, and the trays were changed weekly until they were screened for infection.

Determination of Infection.

Daily observations were made for mortality throughout the experimental period (70 days). Snails were individually examined for the presence of infection at 21 days after exposure and thereafter at intervals of 7 days. To check for infection, snails were placed under total darkness for eight hours. Thereafter, the snails were placed in a 2.0 X 3.0 cm glass vial containing 4.0 ml of filtered aerated tap water, exposed to bright illumination for three hours and examined for the presence of cercariae using a dissecting microscope. Snails found to be initially negative for infection were also examined at intervals of 7 days. At the end of the experimental period those snails which were never found to be positive for infection were crushed and their digestive glands examined for the presence of sporocyst development.

According to the available data obtained from the periods of infection divided in 0-28 days (pre-patent) and 29-70 days (patent), the snail mortality and the percentage of snails shedding cercariae we could evaluate the susceptibility of **B. glabrata** to different strains of **S. mansoni**.

The infection with sympatric strains of Puerto Rican snails and **S. mansoni** will be study in future works.

RESULTS AND DISCUSSION

The data obtained is shown in Table 1. It was confirmed that snails failing to shed cercariae did not contain schistosome larval stages.

TABLE 1
Susceptibility of **Biomphalaria glabrata** from Brazil and Puerto Rico to Different Strains of **Schistosoma mansoni**

Snail Strain	Schistosome Strain	Days of Infection							
		0 — 28 days				29 — 70 days			
		Deaths		Shedding Cercariae		Deaths		Shedding Cercariae	
		Nº	%	Nº	%	Nº	%	Nº	%
Brazilian	BH	4	8	0	0	21	42	41	82
	MAP	1	2	11	22	11	22	29	58
	H-30	0	0	0	0	1	2	8	16
	MPROL	0	0	0	0	1	2	5	10
	MPROXR	0	0	0	0	1	0	4	8
	Control	0	0	0	0	0	0	0	0
Puerto Rican	BH	0	0	0	0	43	86	45	90
	MAP	1	2	4	8	17	34	46	92
	Control	1	2	0	0	0	0	0	0

Obs: each exposure group contained 50 snails.

The results show the occurrence of a slower rate of development as well as lower infectivity of the Puerto Rican derived strains of **S. mansoni** than the two Brazilian schistosome isolates for the Brazilian strain of snail.

These differences may indicate that a significant divergence in properties of the two schistosome strains has occurred. Despite these differences, infections did occur with the drug resistant strains suggesting that resistance could travel with the movement of people who were not cured following therapy. Movement of breeding stock has been suggested as one method in the spread of drug resistant ovine nematodes[4]. By contrast to the lowered susceptibility of the Brazilian snails to the Puerto Rican **S. mansoni**, the Puerto Rican snails were much more susceptible to the Brazilian **S. mansoni**.

It would be of interest to know how fast the drug resistant strains of **S. mansoni** from Puerto Rico would adapt to the Brazilian snails if transmission only occurred through this strain of snail. It would also be relevant do determine how fresh isolates from patients from the two geographic regions differ in their infectivity to the two strains of snails. The difference in infectivity reported here might represent long term passage through mice in the laboratory. It is known that passage through mice can affect biochemical properties of the worms[3, 11]. The infectivity of recently isolated drug resistant strains of **S. mansoni** in Brazil should be investigated further for their infectivity to a range of potential intermediate hosts, both within Brazil and from other countries.

RESUMO

Suscetibilidade de linhagens de Biomphalaria glabrata **a cepas de** Schistosoma mansoni

Com duas linhagens de **Biomphalaria glabrata** foi estudada a suscetibilidade de cinco cepas de **Schistosoma mansoni** resistentes e suscetíveis a esquistossomicidas. Três cepas do trematódeo oriundas de Porto Rico apresentaram desenvolvimento mais lento e menor índice de infecção em **B. glabrata** brasileira quando comparados com o comportamento de duas cepas de **S. mansoni** provenientes do Brasil. Por outro lado, as cepas brasileiras do parasita desenvolveram bem e infectaram mais de 90% dos exemplares de **B. glabrata** portorriquenhos. Entre os resultados, ressalta-se que cepas resistentes a esquistossomicidas poderão ser introduzidas por pacientes em diferentes áreas geográficas como Brasil e Porto Rico.

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