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Susceptibility of Wild Populations of *Biomphalaria* spp. From Neotropical South America to *Schistosoma mansoni* and Interference of *Zygocotyle lunata*

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ABSTRACT: Populations of *Biomphalaria straminea*, *Biomphalaria peregrina*, *Biomphalaria tenagophila*, *Biomphalaria orbignyi*, and *Biomphalaria oligoza* from different Argentine localities were exposed to miracidia of *Schistosoma mansoni* EC strain, and *Biomphalaria tenagophila*, in addition to the SJ2 strain. *Biomphalaria straminea* and *B. tenagophila* displayed different susceptibility and compatibility (Frandsen's total cercariae production index class 0–II), whereas *B. orbigny* and *B. oligoza* were incompatible. Although *B. peregrina* and *B. tenagophila* were found naturally infected with the amphistome *Zygocotyle lunata*, all 5 species could be experimentally infected with *Z. lunata*. Exposure to *Z. lunata* infections with *S. mansoni* were obtained in natural populations of *B. straminea* and *B. tenagophila* with the EC strain (13.5–17.1% and 1.2%), respectively, and in *B. tenagophila* with the SJ2 strain (2.6%), 60 days postexposure [PE]), and in *B. orbignyi* and *B. oligoza* (31.1% and 26.7% 60 days PE, respectively, including single infections with *S. mansoni* and double infections with *Z. lunata*). The high susceptibility of *B. orbignyi* and *B. oligoza* is noteworthy, as these 2 species are considered resistant to *S. mansoni*.

Human schistosomiasis was probably introduced repeatedly from Africa to the Americas over the last 500 yr through the slave trade (Despres et al., 1993). In Brazil, schistosomiasis was first detected in the State of Bahia, from where it spread north, south, and southwest over the country; in these areas, natural infection with *Schistosoma mansoni* has been identified in the intermediate hosts *Biomphalaria glabrata*, *Biomphalaria straminea*, and *Biomphalaria tenagophila* (Paraense, 1997). In Argentina, no cases of schistosomiasis have been reported to date, despite the common presence of *B. straminea* and *B. tenagophila* in the northern and northeastern provinces. In addition, information on their susceptibility to *S. mansoni* is scarce (Pellegrino et al., 1968; Borda and Pellegrino, 1976; Paraense and Correa, 1978; Borda and Rea, 2007, 2010).

Zygocotyle lunata was found parasitizing *Anas sibilatrix* Poepping (Sutton and Lunaschi, 1987) and *Cygnus melancorypha* (Molina, 1782) (Digiani, 1997) in Argentina. *Biomphalaria peregrina* and *B. tenagophila* were naturally infected with *Z. lunata*, and susceptibility of other *Biomphalaria* species to this parasite was demonstrated experimentally (Ostrowski de Núñez et al., 2003, 2011). In North America, only species of *Helisoma* are known to act as intermediate hosts (Willey, 1941; Etges, 1992; Nollen, 1994).

Many studies have been performed to determine whether the co-occurrence of trematode larvae other than *Schistosoma* spp. affect interaction between these parasites and their intermediate hosts by interfering with either parasite development in the snail (Lim and Heyneman, 1972, and references therein), or natural resistance of the snail to the parasite (Lie et al., 1977; Southgate et al., 1989). In the present article, we extended these studies to *Z. lunata,* and examined the susceptibility of different populations of *Biomphalaria* spp. to *S. mansoni* from localities in Argentina and Uruguay near the borders with Brazil.

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Wild populations of *Biomphalaria oligoza, Biomphalaria orbignyi, Biomphalaria peregrina, B. straminea,* and *B. t. tenagophila* were collected from the following localities in Argentina: Buenos Aires City (artificial ponds in the Zoological Garden and parks, 34°36′S, 58°27′W); Córdoba Province (Los Dos Rios, 30°58′05″S, 63°58′57″W); Corrientes Province (Alvear, 21°05′99″S, 56°33′12″W; Bonpland, 29°49′02″S, 57°25′41″W; Curuzú Cuatiá, 29°47′32″S, 58°02′59″W; Felipe Yofre, 29°06′04″S, 58°20′31″W; Mburucuyá, 28°02′44″S, 58°13′38″W; Riachuelo, 27°34′59″S, 58°44′42″W; Rio Aguapey, 29°05′00″S, 56°35′58″W; San Miguel, 27°59′31″S, 57°35′21″W; San Roque, 28°34′25″S, 58°42′44″W; Soto, 28°16′14″S, 58°38′21″W; Triangulo, 27°29′17″S, 58°38′21″W); Entre Rios Province (Ayui, 31°16′01″S, 57°59′00″W); La Rioja Province (Patquia, 30°02′32″S, 66°52′46″W); Chubut Province (Cholila, 42°30′42″S, 71°26′49″W); and Espinillar, 33°27′54″S, 54°12′03″W and Salto, 31°27′43″S, 57°06′04″W in Uruguay.

Snails were transported alive to the laboratory and identified by means of comparative morphology based on the reproductive organs and shells, according to Rumi (1991). Snails were placed in 10-ml vials containing dechlorinated tap water and exposed to an artificial light source during 7 days to detect natural infections of any type of cercaria. Snails resulting negative for cercariae emergence were separated in groups of 12–15 specimens and raised in 3-L containers to obtain offspring for experiments. They were kept under controlled laboratory conditions at 26–28 C and fed lettuce ad libitum.

Specimens of *B. glabrata* from wild-caught populations for starting cultures and infected with *S. mansoni* strains SJ2 and EC were introduced in our laboratory from the Instituto Oswaldo Cruz (Rio de Janeiro, Brazil). The life cycle was maintained in CF1 mice following Lewis et al. (1986). Snails used in the experiments were individually placed in vials containing 2.5 ml dechlorinated tap water at 25–27 C and exposed to a counted number of miracidia for 6 hr. Then, 12–15 snails were transferred to 3-L aquaria, and maintained as indicated above. At 30 days postexposure (PE), snails were individually placed under a light source and monitored for cercariae emergence.

The degree of compatibility between *S. mansoni* and the snail populations was assessed using the index of total cercariae production (TCP), calculated as the cercariae production during snail's lifespan per 100 exposed snails (Frandsen, 1979a). The TCP index was estimated for offspring of wild populations of *Biomphalaria* spp. infected with *S. mansoni*. Snails were tested individually, except for the high number of infected snails of *B. tenagophila* populations from Alvear, Ayui, and Triangulo, where groups of 4 snails were tested together. To facilitate counting of cercariae using a stereoscopic microscope, snails were removed and a drop of chlorhydric carmine was added to the water, which was then absorbed on blotting paper. Counting was done 3 times per week, over 180 days.

Biomphalaria peregrina snails naturally infected with *Z. lunata* were collected from a pond at the Zoological Garden of Buenos Aires City. Emerging cercariae encysted immediately on the walls of the container, snail feces, or plants, and ovigerous adults were obtained from mice after oral infection with 10–12 metacercariae. The life cycle of *Z. lunata* in the laboratory started with eggs from these mice and was maintained by

TABLE I. Experiment 1a: *Biomphalaria straminea*, *Biomphalaria tenagophila*, *Biomphalaria peregrina*, *Biomphalaria orbignyi*, and *Biomphalaria oligoza* populations exposed to 6–8 miracidia of *Schistosoma mansoni* EC and SJ2 strains; 60 days PE.*

Collection sites	No. of snails			Daily cercarial production (X ± SD)	Total cercariae production index (class)
	Exposed	Survivors (m)	Infected (%)		
Biomphalaria straminea†					
Ayui	192	188 (2.1)	1 (0.5)	43 ± 18.7	985.4 (I)
Triangulo	480	471 (1.9)	7 (1.5)	65.0 ± 27.2	2,542.9 (I)
Bonpland	96	88 (8.3)	0	–	–
Mburucuyá	150	139 (7.3)	2 (1.4)	70.5 ± 20.5	5,688.7 (I)
Espinillar	346	327 (5.5)	10 (3.1)	89.5 ± 50.4	11,754.9 (II)
San Miguel	245	196 (20.0)	0	–	–
Soto	105	100 (4.8)	0	–	–
Riachuelo	230	218 (5.2)	0	–	–
Rio Aguapey	96	95 (1.0)	0	–	–
Biomphalaria tenagophila‡					
Alvear	484	478 (1.2)	19 (4.0)	84.2 ± 55.1	14,767.6 (II)
Ayui	770	748 (2.9)	40 (5.3)	103.5 ± 75.1	27,081.8 (II)
Curuzú Cuatiá	166	163 (1.8)	4 (2.5)	52.5 ± 28.3	6,098.8 (I)
F. Yofre	216	207 (4.2)	3 (1.4)	56.7 ± 28.6	2,207.4 (I)
San Roque	630	591 (6.2)	0	–	–
Triángulo	1240	1,160 (6.5)	94 (8.1)	93.8 ± 71.9	32,506.4 (II)
Soto	218	216 (0.9)	7 (3.2)	79.9 ± 47.8	11,530.3 (II)
Bonpland	360	351 (2.5)	0	–	–
Mburucuyá	210	159 (24.3)	0	–	–
Salto	192	188 (2.1)	0	–	–
Biomphalaria peregrina†					
Zoological Garden	120	116 (3.3)	0	–	–
Cholila	96	90 (4.2)	0	–	–
Biomphalaria orbignyi†					
Patquia	80	77 (8.3)	0	–	–
Biomphalaria oligoza†					
Dos Rios	60	55 (3.8)	0	–	–

* m, mortality (%); $\bar{x}$, average; SD, standard deviation.
† *Schistosoma mansoni* EC strain.
‡ *Schistosoma mansoni* SJ2 strain.

infection of laboratory-reared snails and successive inoculation in mice, as described by Ostrowski de Núñez et al. (2003).

To obtain large numbers of miracidia in a short time for experimental infections, eggs with developed miracidia were stored at 10 C until a sufficient number were available. They were then exposed in groups to light at 26 C and emerging miracidia were placed with snails within 90 min.

Infection experiments involved laboratory-reared snails (juveniles 2–3 mm in diameter) from wild-caught populations of *B. peregrina, B. straminea, B. oligoza, B. tenagophila*, and *B. orbignyi*. Tables I and II indicate the strain of *S. mansoni* used in each experiment. About 50 snails from each species were not exposed to *S. mansoni* or *Z. lunata* and were used as control groups to compare mortality.

Snails placed individually in 5–10 ml dechlorinated tap water were subjected to 1 of the following experiments. Single exposure: (1a) exposure to 6–8 miracidia of *S. mansoni*, and (1b) exposure to 5 miracidia of *Z. lunata*. The double-exposure trial (2a; 2b) involved *B. orbignyi* (Riachuelo), *B. oligoza* (Los Dos Ríos), and populations of *B. straminea* and *B. tenagophila* (Triangulo and San Roque, respectively), but not *B. peregrina*, because of its small number of offspring. (2a) Exposure to *Z. lunata* and *S. mansoni*. Exposure to 5 miracidia of *Z. lunata*, followed by exposure to 5 miracidia of *S. mansoni* 24 hr later. In 2b, there were 2 consecutive exposures to 5 miracidia of *S. mansoni* separated by a 24-hr interval (control).

After overnight exposure, all snails were maintained in aquaria at 24–26 C and fed with lettuce ad libitum; water was changed once a week. Snails were isolated individually from day 15 onward at different times PE and checked for cercariae emergence.

Experiments 2a and 2b were performed simultaneously. Only snails that shed cercariae were considered to be infected following Frandsen (1979b). The percentage of infection was determined for live snails at day 45 of the experiment 1b and at day 60 PE of the experiments 1a, 2a, and 2b. The mortality for all the control groups ranged between 2.0 and 6.0% (not included in tables); mortality of exposed snails are given in the tables.

In a single exposure to 6–8 miracidia of *S. mansoni* (Experiment 1a; Table I), *B. straminea* from 4 of 9 localities became positive after exposure to the EC strain of *S. mansoni*, but at a low percentage (0.5–3.1%), with compatibility classes of I–II. The prepatent period varied between 38 and 49 days PE, with exposed snails showing low mortality (1.0–8.3%), except for snails from San Miguel, which reached 20.0%.

Biomphalaria tenagophila from 6 of 10 localities were infected after exposure to the SJ2 strain of *S. mansoni*, with infection percentages varying over a relatively wide range (1.4–8.1%) and compatibility classes of I–II. The prepatent period in all groups varied between 36 and 49 days PE and mortality was low (0.9–6.5%), except for snails from Mburucuyá, which reached 24.3%.

In total, 120 *B. peregrina* from the Zoological Garden of Buenos Aires City, and 96 from Cholila (Chubut Province) were negative after exposure to the EC strain of *S. mansoni*. Overall, 48 *B. oligoza* and 48 *B. orbignyi* were negative after exposure to the EC strain of *S. mansoni*. Low mortality percentages (3.3–8.3%) were observed for exposed *B. peregrina*, *B. oligoza*, and *B. orbignyi*, similar to those in the respective control groups.

TABLE II. Experiment 1b: **A**. Exposure to 5 miracidia of *Zygocotyle lunata* (*Z.l.*) after 45 days PE. Experiments 2a; 2b: **B.** Exposure to 5 miracidia of *Z.l.* followed by exposure to 5 miracidia of *Schistosoma mansoni* (*S.m.*) separated by a 24-hr interval. **C**. Two consecutive exposures to 5 miracidia of *S.m.* EC strain separated by a 24-hr interval.*

	A. Ostrowski de Núñez et al. (2003)		B. *Z.l.*–*S.m.*					C. *S. m.*–*S. m.*	
Species (collection site)	*N* (m)	*Z.l. n* (%)	*N*† (m)	*Z.l.* *n* (%)	*S.m.* *n* (%)	*Z.l.* + *S.m.* *n* (%)	*Z.l.* + (*Z.l.* + *S.m.*) *n* (%)	*S.m.* + (*Z.l.* + *S.m.*) *n* (%)	*N*† (m)	*n* (%)
B. straminea‡ (Triangulo)	100/96 (4.0)	70 (73.0)	120/104 (13.3)	70 (67.3)	5 (4.8)	9 (8.7)	79 (76.0)	14 (13.5)	100/96 (4.0)	1 (1.0)
B. straminea‡ (Triangulo)			253/211 (16.6)	113 (53.6)	16 (7.6)	20 (9.5)	133 (63.0)	36 (17.1)	–	–
B. straminea‡(Ayui)			373/315 (15.5)	183 (58.1)	21 (6.7)	29 (9.2)	212 (67.3)	50 (15.9)	96/82 (14.6)	0
B. tenagophila‡ (San Roque)			90/82 (8.9)	32 (39.0)	0 (0)	1 (1.2)	33 (40.2)	1 (1.2)	72/69 (4.2)	0
B. tenagophila§ (San Roque)	40/34 (15.0)	18 (53.0)	120/115 (4.2)	53 (46.1)	1 (0.8)	2 (1.7)	55 (47.8)	3 (2.6)	120/113 (5.8)	0
B. orbignyi‡ (Patquia)	48/45 (6.3)	36 (80.0)	48/45 (6.3)	13 (28.9)	4 (8.9)	10 (22.2)	23 (51.1)	14 (31.1)	48/45 (6.3)	0
B. oligoza‡ (Los Dos Rios)	15/15 (0)	3 (20.0)	48/45 (6.3)	0	8 (17.8)	4 (8.9)	4 (8.9)	12 (26.7)	48/47 (2.1)	0
B. peregrina Zoological Garden	20/19 (5)	4 (21.1)	–	–	–	–		–	–	–

* *Z.l.* + (*Z.l.* + *S.m.*): snails producing cercariae of *Z.l.* only and cercariae of both species. *S.m.* + (*Z.l.* + *S.m.*): snails producing cercariae of *S.m.* only and cercariae of both species. m, mortality (%); *N*, number of snails exposed/survived; *n*, number of snails infected. Percentages calculated on surviving snails.

† 60 days postexposure.

‡ *Schistosoma mansoni* EC strain.

§ *Schistosoma mansoni* SJ2 strain.

Infection percentages for *B. straminea, B. tenagophila, B. orbignyi, B. oligoza,* and *B. peregrina* exposed to the miracidia of *Z. lunata* (Experiment 1b; Table II, A) were 73.0%, 53.0%, 80.0%, 20.0%, and 21.1%, respectively, as reported by Ostrowski de Núñez et al. (2003). Mortality was low (0–6.3%) for all species, except for *B. tenagophila* (15.0%).

Populations of *B. straminea* and *B. tenagophila*, and *B. oligoza* and *B. orbignyi*, which were low, or nonsusceptible, to *S. mansoni*, could be successfully infected after exposure to miracidia of *Z. lunata* (Experiment 2a; Table II, B). *Biomphalaria tenagophila* was exposed to the SJ2 and EC strain; the other 3 species were exposed to the EC strain of *S. mansoni* only.

Two groups of the Triangulo *B. straminea* population and 1 of the Ayui population were exposed. The mean prepatent period was 27 days and 34 days for *Z. lunata* and *S. mansoni*, respectively, with the first snail releasing both cercariae at day 38 PE. The mortality in these groups was higher than in the others (13.3–16.6%).

The prepatent period for *Z. lunata* in *B. tenagophila* was 24–27 days; 1 snail was positive for *S. mansoni* 40 days PE, and 2 released both cercariae 49 days PE. Snails infected with the SJ2 strain showed low mortality (4.2%), whereas in those infected with the EC strain the mortality was higher (8.9%).

On day 60 PE, 10 *B. orbignyi* produced cercariae of both species, while 13 snails produced only cercariae of *Z. lunata* and 4 produced only cercariae of *S. mansoni*. The prepatent period was 28–35 days for *Z. lunata* and 35 days for *S. mansoni*; double emission was first detected 49–56 days PE. The mortality of these snails reached 6.3%.

Release of *S. mansoni* cercariae from *B. oligoza* was first detected in 1 snail on day 35 PE. On day 60, cercariae of *S. mansoni* were produced by 12 snails, 4 of which also released cercariae of *Z. lunata* (prepatent period: 55 days). This group exhibited low mortality (6.3%).

In Experiment 2b (Table II, C), the populations of *B. tenagophila* (San Roque), *B. oligoza*, and *B. orbignyi* were negative, whereas 1 of 100 (1.0%) *B. straminea* from Triángulo became infected. Mortality was low (2.1–6.3%) for all groups, except for *B. straminea* from Ayui, which reached 14.6%. Earlier studies on the susceptibility of different *B. tenagophila* populations from Argentina and Uruguay to strains of *S. mansoni* rendered negative results (Pellegrino et al., 1968; Borda and Pellegrino, 1976; Paraense and Correa, 1978), but later studies reported susceptibility, in some cases, with compatibility classes II and III (Borda and Rea, 2007, 2010). In the present study, testing the susceptibility of populations of different *Biomphalaria* species to *S. mansoni*, 8 of the 15 studied populations of *B. tenagophila* and *B. straminea* were positive, with TPC index of class I and II, while those of *B. peregrina*, *B. oligoza*, and *B. orbignyi* were negative. Snails were collected over a wide area drained by Paraná and Uruguay Rivers from localities other than those reported by Borda and Rea in the studies cited above. On this basis, their results, together with those obtained here, would be representative of all the *Biomphalaria* populations in the Argentinean region of major risk for the expansion of schistosomiasis.

Experimental data from the literature reveal highly variable infection percentages for *B. straminea* and *B. tenagophila*, with the former generally having lower values (Souza, 1986; Souza et al. 1995, and references therein). Paraense and Correa (1989) reported a considerably higher susceptibility for *B. straminea* aff. *straminea* (species inquirenda) from Espinillar (Uruguay), than that obtained in this study (23.0% vs. 3.1%, respectively).

None of the *B. peregrina* snails exposed to miracidia of *S. mansoni* became infected, in agreement with Souza et al. (1988), but not with Paraense and Correa (1973), who succeeded in infecting 33.0% of the *B. peregrina* collected from the State of Parana (Brazil), and 15.0% near Quito (Ecuador) with 2 different strains of *S. mansoni*.

When considering snails producing cercariae of *S. mansoni* only and of both *S. mansoni* and *Z. lunata*, the nonsusceptible populations of *B. tenagophila* from San Roque (1.2% for the EC strain, 2.6% for the SJ2 strain), the less-susceptible population of *B. straminea* from Triangulo

(13.5%), and the species considered naturally resistant, *B. oligoza* (26.7%) and *B. orbignyi* (31.1%), were positive for *S. mansoni* after infection with *Z. lunata* (Table IIB). Unfortunately, these experiments could not be repeated because of the small number of offspring of *B. oligoza* and *B. orbignyi*, the collapse of *Z. lunata* life cycle after 5 yr in the laboratory (possibly caused by inbreeding), and the impossibility of finding naturally infected snails to continue the life cycle (Ostrowski de Núñez et al., 2011)

Experiments with resistant strains of *B. glabrata* making them susceptible to *S. mansoni* after infection with different species of echinostomatids were performed by Lie et al. (1977, 1983). There are many examples of snails becoming susceptible to a trematode species when previously infected with larval stages of another one. This was reported for *Bulinus tropicus* with *S. bovis* following primary infection with *Calicophoron microbothrium* (see Southgate et al., 1989) and for the prosobranch *Velacumantus australis* with *Austrobilharzia terrigalensis* (see Walker, 1979). Moreover, the presence of larval stages of *S. mansoni* in snails that were later infected with another trematode species was found to be related to a sharp decrease in cercariae infectivity and worm recovery in mice (Jourdane et al., 1990).

Snails parasitized by *Z. lunata*, or by 2 different trematode species, are rare in nature. *Zygocotyle lunata* was found in none of the *Biomphalaria* spp. collected in extensive sampling performed in Corrientes Province (Ostrowski de Núñez et al., 2003) or across the distribution range of the snails (data not shown). Recently, the first natural infection of *B. tenagophila* by *Z. lunata* was reported in Salta Province (Ostrowski de Núñez et al., 2011).

No direct relationship between susceptibility levels of the snail populations and human infection prevalence has been found in areas that are endemic for schistosomiasis. In the present study, *B. straminea* and *B. tenagophila* showed low levels of compatibility, but possibly sufficient for sustaining high levels of prevalence of the infection in the human population. In northeastern Brazil, where *B. straminea* is the only vector, its prevalence of *S. mansoni* infection is about 1.0%, whereas that in the human population is higher than 50.0% (Paraense, 1997).

The susceptibility and high prevalences that can be reached by *B. orbignyi* and *B. oligoza* to *S. mansoni* following primary infection with *Z. lunata* may be of interest for the transmission of schistosomes; however, this possibility seems unlikely because of the uncommon occurrence of double infections in nature.

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