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Compatibility of *Biomphalaria glabrata* and *B. alexandrina* snails to infection with an egyptian strain of *Schistosoma mansoni* through two cycles in the experimental final host

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ABSTRACT

The infection rate and cercarial production from *B. glabrata*, in comparison with *B. alexandrina* snails, post their exposure to *S. mansoni* miracidia of an Egyptian strain after two cycles in albino-mice was studies.The results indicated that infection rate of *B. glabrata* with the Egyptian strain of *S. mansoni* was less than that of *B. alexandrina* snails On the other hand infected *B. glabrata* exhibited a longer life span and a higher number of shedding cercariae, It was also, noticed that in the first cycle mice infected with *S. mansoni* cercariae shed from infected *B. alexandrina* snails, the mean number of worms recovered from infected mice was approximately twice that in mice infected with cercariae shed from infected *B. glabrata* snails The same observation was recorded from the mean number of ova/g liver tissue from infected mice. In the second cycle the same observation was recorded as first cycle suppression in the infection rate of *B. glabrata* than that *B. alexandrina*. Also, longer prepatent period and life span Also, mice infection as the number of worms per infected mouse by cercariae shed from *B. alexandrina* snails was approximately 2.5 times that of mice infected by cercariae shed from *B. glabrata* being 29.3 and 12.5 worms/mouse.The results also indicated that the egg laying capacity of B. glabrata was higher than that *B. alexandrina*, It is concluded from this work that infectivity of *S. mansoni* cercariae shed from *B. glabrata* snails after two cycles of mice infection and used to infect the experimental final host was less than that of cercariae shed from infected B. alexandrina snails. This may declare a low compatibility of *B. glabrata* snails with the Egyptian strain of *S. mansoni* in comparison with B. alexandrina snails. However, this conclusion needs more passages of mice infection with cercariae to have precise data and conclusions.

Keywords: *Biomphalaria alexandrina*; *B. Glabrata*; *Schistosoma mansoni*; Compatibility

1. INTRODUCTION

Schistosomiasis is one of the major health problems in many developing countries [1]. The prevalence of this parasite in human population depends on the number of infected snails in an area. The specificity of parasite-host interactions has received great attention by parasitologists and evolutionary biologists [2].

It was known till 1996 that *B. alexandrina* is the only planorbid species acting as the intermediate host of *Schistosoma mansoni* in Egypt. This snail was widely distributed now along the Nile Delta and valley. Recently, *B. glabrata* the intermediate host of *S. mansoni* in the New world, has been reported from natural freshwater habitats in Egypt [3]. The authors collected the snail population from many water courses of irrigation and drainage systems in Qalyoubia and Kafr El-Sheikh Governorates.

In trematode-snail interactions, which are generally regarded as highly specific [4], compatibility patterns of species or strains of parasites and hosts have been used for phylogenetic studies as well as for investigations of parasite-host convolution on a local scale [5], Especially in medically relevant blood flukes. Snail compatibility is also a topic of practical importance for epidemiological surveys and development of biological control methods. This is the reason why most work on snail compatibility and the snail's internal defense system against trematode infections was performed with *S. mansoni* [6]. Host snails of the genus Bomphalaria respond with humoral and cellular mechanisms to *S. mansoni* sporocysts [7]. It was recently demonstrated by selection experiments under laboratory conditions with different snail lines that, in the system *S. mansoni*, *B. glabrata*, compatibility characteristics seem to be inherited with resistance being dominant over susceptibility [4,8].

In fact, we found that an Egyptian (laboratory) strain of S. mansoni discriminated between its host snail *B. alexandrina* and other species and strains, already during approach and after contac [9,10].

The present study aims to study the compatibility of *B. glabrata* snails to infection with an Egyptian strain of *S. mansoni* which is very important from the epidemiological point of view and draws the attention to the possible role of this snail (*B. glabrata*) may play in schistosomiasis in Egypt.

2. MATERIALS AND METHODS

2.1. Snails

The snails used in the present study, *Biomphalaria alexandrina* and *B. glabrata*, were obtained from the Schistosome Biological Supply Centre (SBSC), Theodor Bilharz Research Institute (TBRI). *B. glabrata* snails were collected from some foci in the irrigation canals at Qalyoubia Governorate [3], transferred to laboratory examined for natural trematode infection, negative and healthy ones were maintained under laboratory conditions [11].

2.2. Snails Infection

S. mansoni miracidia of the Egyptian strain were obtained from SBSC. *B. alexandrina* and *B. glabrata* snails used for infection were almost the same size (4 mm - 5 mm) and individually exposed to miracidia (10 miracidia/snail) for 24 hrs after miracidial exposure, snails were maintained in dechlorinated water at 24˚C ± 1˚C then from 20 days post exposure, samples from surviving snails were microscopically examined for sporocysts. The other surviving snails were examined to cercarial shedding after 30 days post miracidial exposure by exposing each snail in 2 ml water for 3 hrs to 100 watt filament lamp, 40 cm far at 26˚C ± 1˚C. Positive snails were isolated, marked and re-exposed for cercarial shedding twice weekly. The cercariae emerged from each positive snail were counted and recorded. The prepatent period, infection rate, periodic cercarial production and life span of infected snails were determined for each species.

2.3. Fecundity of *B. alexandrina* and *B. glabrata*

For each snail species three replicates were used each of 10 snails (5 mm - 8 mm)/L the aquaria were provided with thin plastic sheets for egg deposition. The snails were fed blue-green algae (*Nostoc muscorum*) and dried lettuce leaves. The egg clutches were weekly collected and eggs were counted and recorded.

2.4. Animals Infections

Laboratory bred albino mice in this study were obtained from SBSC cercariae shed from infected *B. alexandrina* snails were used to infect a group of 6 mice and those shed from *B. glabrata* were used to infect another 6 mice group. The infection was done by the tail immersion method (100 cercariae/mouse [11] and 4 mice non-infected act as a control.

2.5. Parasitological Investigations

Worm load and distribution were studied in infected mice by perfusion (hepatic and intestinal) method and the number of eggs was counted per g tissue [12]. The S. mansoni eggs from mice infected with cercariae shed from *B. alexandrina* snails were hatched to miracidia that used to infect clean groups of *B. alexandrina* and *B.glabrata* snails. The same re-exposure of snail to miracidia was followed by miracidia hatched from *S. mansoni* eggs obtained from mice infected with cercariae shed from *B. glabrata* snails. This technique of re-exposure of snail to miracidia was repeated after another passage of mice infection with cercariae shed from the first passage of infection.

2.6. Student's t-Test

Student's t-Test and chi-square test [13] were used in comparing the means and rates of experimental group statistically.

3. RESULTS AND DISCUSSION

3.1. First Cycle

Laboratory produced snails *B. alexandrina* and *B. glabrata* were exposed to *S. mansoni* miracidia individually (10 miracidia/snail). The result indicated that after 20 days from exposure to miracidia the survival rate of *B. glabrata* significantly higher (96%) than *B. alexandrina* (76%). Crushing ten snails from each species to examine for sporocyst revealed that positive snails of *B. alexandrina* was 8 snails, however only one snail positive of *B. glabrata*.

The present results showed that there are significant differences between *B. alexandrina* and *B. glabrata* infected with *S. mansoni* from Egypt, in the tested parameters (survival rate, infection rate, prepatent period, cercarial production, duration of shedding and life span of infected snails).

From **Table 1** the results indicated that after 34 days from miracidial exposure the survival rate of *B. glabrata* was higher about 70% compared to 46% of *B. alexandrina* with significant difference ($p < 0.001$). These results agree with [7,14], they found that a reduction in the survival rate of infected B. alexandrina compared to infected B. glabrata snails through the prepatent period.

The present results showed that *B. glabrata* was less susceptible to infection with Egyptian strain of *S mansoni* than *B. alexandrina* being 8.6% and 78.3% respectively.

The infection rate was significantly much higher of

Table 1. Survival rate, infection rate, prepatent period, cercarial production, duration of shedding and life span of infected *B. alexandrina* and *B. glabrata* with *S. mansoni* miracidia.

B. glabrata	B. alexandrina	op
50	50	Number of exposed snails to miracidia of Egyptian strain
35	23	Number of survived snails at first cercarial shedding
70%	46%	Survival rate
3	18	Number of infected snails
8.6%	78.3%	Infection rate
		Prepatent period (days)
59 - 61	34 - 53	Range
60.0 ± 1.0	37.5 ± 5.8	Mean ± SD
11492	14731	Total number of cercariae
1888 - 7482	21 - 2485	Range
3830.7 ± 316	818.4 ± 720.8	Mean ± SD
		Duration of cercarial shedding (days)
30 - 69	4 - 45	Range
43.3 ± 22.2	18.1 ± 11.2	Mean ± SD
		Life span (days)
71 - 110	47 - 86	Range
97 ± 22.5	56.8 ± 9.1	Mean ± SD

B.alexandrina than that of *B.glabrata* ($p < 0.001$). This agrees with the previous findings of Files [15], Kuntz [16] and Cridland [17] they found that the susceptibility of *B.glabrata* to *S mansoni* from Egypt was very low being 8% - 30% and 0%. The same conclusion was recorded by Yousif *et al*. [18]. They found that the infection rate of all Egyptian *S. mansoni* strains was significantly higher in *B. alexandrina* than each of *B. glabrata* and the hybrid snail. Also, Kalbe *et al*. [9] found that infection rate of Brazilian snail *B. glabrata* with *S. mansoni* Egyptian strain ranged between 12.3% and 18.7% Bakry [7] found that infection rate of *B. glabrata* and *B. alexandrina* from Domietta 8% and 16% respectively were significantly less than those of *B. alexandrina* from Fayoum and Giza (56% and 64%, respectively $p < 0.001$)

The results showed that the prepatent period of *S. mansoni* in *B. glabrata* was significantly longer than in *B. alexandrina* being 60 days and 37.5 days respectively ($p < 0.001$). This result agrees with Yousif *et al*. [3] who showed that *B. glabrata* has significantly longer incubation period than *B. alexandrina* being 33.45 days and 28 days respectively ($p < 0.05$) when exposed to laboratory strain of *S. mansni* (SBSC) from Egypt.

The cercarial production of *B. alexandrina* was significantly less ($p < 0.001$) than that of *B. glabrata* the mean number of cercariae per snail of infected *B. alexandrina* was 818.4 cercariae/snail compared to mean number of cercariae shed from infected *B. glabrata* was 3830.7 cercariae/snail.

The result agrees with Yousif *et al*. [18] who found that cercarial production from *B. alexandrina* was lower than those from *B. glabrata* and hybrid snail species post their exposure to an Egyptian strain from SBSC. This observation is in parallel with that of Frandsen [19] who found that *B. glabrata* infected with various strains of *S. mansoni* from St. Lucia and the West Indies produced variable numbers of cercariae. However Bakry [7] found that the cercarial production of *B. glabrata* and *B. alexandrina* from Damietta was significantly lower than that of B. alexandrina from Fayoum and Giza.

The result indicated that the mean of duration of shedding and life span of infected *B. glabrata* was longer than of *B. alexandrina* infected snails being 43.3 and 97 days compared to 18.1 and 56.8 days respectively ($p < 0.001$). A similar result was observed by yousif *et al*. [18] who found that duration of cercarial shedding from *B. alexandrina* infected with *S. mansoni* from Egypt was shorter than those of *B. glabrata* and hybrid snails. Frandsen [19] who found that the longest duration of cercarial production by *B. glabrata*, St.Lucia infected with its local *S. mansoni* strain was 180 days.

The infected mice by cercariae of *B. glabrata* and cercariae of *B. alexandrina* were dissected after 60 days from exposure to cercariae by perfusion hepatic and intestine method. The worm were picked out and placed in normal saline and counted. The number of ova/g tissue liver was estimated (**Table 2**) show that the number of infected mice by cercariae of *B. alexandrina* was 5 mice and the other infected with cercariae from *B. glabrata* was 4 mice. From **Table 2** it was indicated that the lowest number of worms was obtained from mice infected with cercariae shed from *B. glabrata* snails being 38 worms compared to 85 worms obtained from mice infected with cercariae shed from *B. alexandrina*. in the first cycle the mean number of worms per infected mouse with *Schistosoma cercariae* shed from *B. glabrata* was reduced being 9.5 worms/mouse compared to 17 worms/mouse infected with cercariae shed from B. alexandrina. This result agrees with the previous findings of Jourdane *et al*. [20] recorded a reduction in the infectivity of *Schistosome cercariae* shed from *B. glabrata* infected with both *S. mansoni* and *E. liei*.

The mean number of ova cunt per g tissue liver of infected mice with cercariae of *B. glabrata* decreased significantly than that of infected mice by cercariae shed ding from *B. alexandrina* being 771.8 compared to 1579

Table 2. Total and mean number of worms in mice infected with *S. mansoni* cercariae from *B. alexandrina* and *B. glabrata* snails (the first passage of mice infection).

Case (1) worm load/mouse infected by cercariae from *B. glabrata*				Case (1) worm load/mouse infected by cercariae from *B. alexandrina*				Mice' number
Total	couple	M	F	Total	Couple	M	F	
5	2	0	1	17	3	7	4	1
13	5	0	3	25	7	3	8	2
16	6	0	4	10	3	4	0	3
4	0	1	3	15	4	5	2	4
-ve	-ve	-ve	-ve	18	6	2	4	5
-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	6
38	13	1	11	85	23	21	18	Total
9.5 ± 5.9	4.3 ± 2.1	1	2.8 ± 1.3	17 ± 5.4	4.6 ± 1.8	42 ± 1.9	3.6 ± 3.0	Mean ± SD
	771.8 ± 99.9				1579 ± 125.8			Mean of ova/g tissue liver

F: Female, M: Male.

ova/g tissue. Warren & Peters [21] obtained adult worms from mice exposed to 40 S. mansoni cercariae with only 10 minutes exposure period. Blumenthal & Jewsbury [22] showed that increasing of cercarial age (2,3,6,8 and 10 hours) gave no effect on percentage worm recovery.

The present results showed that the number of ova retained in the tissue of liver increased with the increase of number of worms. Similar relation was reported by Kloetzel [23] and Koura [24] who found that the number of retained eggs was always proportional to the worm burden. On the contrary, Grove & Warren [25] stated that with increasing worm burden, the number of eggs in the liver per worm pair tended to decrease. The *S. mansoni* eggs from mice infected in the first cycle with cercariae shed from *B. alexandrina* snails were hatched to miracidia that used to infect group 1) clean *B. alexandrina* and group 2) clean *B. glabrata*. Also, miracidia hatched from eggs from mice infected with cercariae shed from *B. glabrata* were used to infect group 3) clean *B. alexandrina* and group 4) clean *B. glabrata*.

The first cycle: the present results showed that there were no difference between the result of first cycle and second cycle. The survival rate was higher in infected *B. glabrata* than that of infected *B. alexandrina*. Also, the infection rate was highest in group 1) being 66.7% compared to 14.3% and 53.3% for group 2) and 3) while group 4) was the lowest infection rate being 10% (**Table 3**).

In the second cycle the mice infected by cercariae shed from each group of snails infected, the number of mice infected in each group was 5 mouse. From T**able 4** the result indicated that the highest mean number of worms/mouse was obtained from mice infected with cercariae shed from *B. alexandrina* group 1) being 29.3 worms/mouse while the mice infected with cercariae shed from snails in group 4) showed a low value, being 12.5 worms/mouse, with a reduction rate of 42.7% from that group 1). This may indicate that infectivity of Schistosoma cercariae of an Egyptian strain shed from B. glabrata snails to albino mice was suppressed by successive passages of mice and snails infection with this parasite strain. Also, the mean number of ova per g tissue of liver in infected mice in group 1) was higher being 1504 compared to 670.5 ova/g in mice infected with cercariae from snail group 4).

In the present study, cohorts of *B. alexandrina* and *B. glabrats* snails were maintained for 4 weeks under standard laboratory conditions at a constant temperature (25˚C + 1˚C) for comparing fecundity of both snail species. *B. glabrata* snails showed a high survival rate (Lx) of 93% compared to 66% of *B. alexandrina*. The net reproduction rate of *B. glabrata* (R_o) was higher than that of *B. alexandrna* after experimental observation period (4 weeks) being 34.7 and 4.69, respectively (**Table 5**). This observation is in accordance with that of Yousif *et al*.[18] who recorded a high value of (R_o) for *B. glabrata* in comparison with that of *B. alexandrina* snails being 1265.9 and 145.3 respectively. However, Sturrock [26] and Pointier *et al*. [27] attributed that difference to strain and type of feeding of the snails.

It is concluded from this work that there is a low compatibility of *B. glabrata* snails with an Egyptian strain of *S. mansoni* in comparison with *B. alexandrina* snails. However, this conclusion needs more passages of mice infection with cercariae to have precise data and conclusions.

Table 3. Survival rate, infection rate, prepatent period, cercarial production, duration of shedding and life span of infected groups of *B. alexandrina* and *B. glabrata* with *S. mansoni* miracidia from mice infected in the first cycle.

Life span (days) Range Mean ± SD	Duration of shedding (days) Range Mean ± SD	Total no of cercariae/snail Mean ± SD	Prepatent period (days) Range Mean ± SD	Infection rate		Survivorship at 1st shedding		Number of groups and snail species
				%	No.	%		
35 - 66	5 - 31	5931	25	66.7	12	60	18	
44.4 ± 11.7	15.5±10.1	1937.2 ± 417.4						1) B. alexandrina
47 - 110	5 - 40	16264	42 - 70	14.3	3	70	21	
83.3 ± 32.2	23.3±17.6	5421.3 ± 1995.9	58 ± 14.4					2) B. glabrata
43 - 53	4 - 22	5860	25 - 31	53.3	8	50	15	
47.8 ± 3.2	13.6 ± 7.2	732 ± 392.5	28.5 ± 2.9					3) B. alexandrina
45 - 70	5 - 14	1255	40 - 53	10	2	66.7	20	
57.5 ± 17.7	9.5 ± 6.4							4) B. glabrata
		627.5 ± 62.9	46.5 ± 9.2					

Table 4. Worm load per mouse infected with *S. mansoni* cercariae shed from snails of *B. alexandrina* and *B. glabrata* in the second cycle.

Mean no of ova/g tissue liver ± SD	Total no. of worms Mean ± SD	Worm load/mouse				No. of infected mice	No. of survived mice	Cercariae shed fom snail groups
		Total	Couple	M	F			
	88	31	6	9	10	3	3	(1)
1504 ± 409.3	29.3 ± 2.1	27	3	6	15			
		30	4	9	13			
567	15	15	4	2	5	1	3	(2)
	41	19	2	9	6	3	4	(3)
773.7 ± 211.6	13.7 ± 4.6	11	2	4	3			
		11	1	3	6			
	25	11	3	2	3	2	4	(4)
670.5 ± 160. 5	12.5 ± 2.1	14	2	3	7			

Table 5. Survival rate (L_x) & fe cundity (M_x) of *B. alexandrina* and *B. glabrata* after four weeks of maintenance under standard laboratory conditions.

B. glabrata			*B. alexandrina*			Observation period (weeks)
L_xM_x	M_x	L_x	L_xM_x	M_x	L_x	
10.5	10.5	1.00	1.2	1.2	1.00	1
10.2	10.2	1.00	0.99	1.1	0.90	2
8.1	8.37	0.96	1.2	1.7	0.73	3
5.9	6.42	0.93	1.3	1.9	0.66	4
	34.7			4.69		R_o

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