Effect of lining Water Bodies on the Infection of *Biomphalaria alexandrina* Snails by *Schistosoma mansoni* Miracidia

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***Abstract*—** Effect of lining water bodies with cement, plastic and mud on the infection of *Biomphalaria alexandrina* by *Schistosoma mansoni* miracidia was studied in the present work. Snails infected by both mass and individual infection then maintained in aquaria with mud substratum produced a higher significant number of cercarial output (6375 and 3361 cercariae, respectively) compared to those maintained in cement (1617 and 74 cercariae, respectively), plastic (1481and1446 cercariae, respectively). control groups represented by 2088 and 3527cercariae respectively. The infection rate for snails infected by mass infection and maintained in aquaria with mud, cement, plastic and control was 81.25%, 75%, 71.43%, 57.89%, respectively. This rate was decreased for those infected by individual infection reaching 78.26%, 50%, 31.58%, 68.18%, respectively. An extremely highly significant reduction ($p<0.001$) in the mean number of cercariae /snail was observed in snails infected by individual infection and maintained in aquaria lined with cement (4.17±4.9) comparing with those in mud, plastic and control which recorded 75.85±45.29, 78.3±57.48 and 65.1±72.3, respectively. A highly significant increase ($p<0.001$) in the percentage mortality among snails infected by mass and individual infection appeared in those maintained in cement substratum which recorded 40%, 66.7%, respectively.

***Keywords*—** *Biomphalaria alexandrina*, lining canals, infection, *Schistosoma mansoni*.

I. Introduction

SCHISTOSOMIASISis is a chronic parasitic disease caused by blood flukes (trematode worms) of the genus *Schistosoma*. Schistosomiasis transmission has been documented in 77 countries, however those at most risk of infection are in 52 countries [1]. The disease continues to spread to new geographic areas. Factors that favour spread include growth in international travel and population migration, and the development of new water resources [2].

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The eggs of *S .mansoni* worms are emitted in the faeces of the final host into the water, the ripe miracidia hatches out of the eggs. The hatching happens in response to temperature, light and dilution of faeces with water. The miracidia search for a suitable freshwater snail *Biomphalaria alexandrina* to act as an intermediate host and penetrate it. Then, the parasite develops via a so-called mother-sporocyst and daughter-sporocyst generation to the cercariae. The purpose of the growth in the snail is the numerical multiplication of the parasite. From a single miracidium result a few thousand cercarie, every one of which is capable of infecting man [3]. One of the environmental modifications for controlling snail vectors is the lining of water canals [**4**]. In China, reference **[5]** stated that Environmental modification was primarily carried out in areas of low endemicity with the aim of permanently interrupting transmission. Environmental modifications included the digging of new ditches and filling of old ones, lining of irrigation canals with concrete, and the alteration of sluice gates to prevent snails from spreading into other sections of an irrigation system. Reference [6] in Egypt, reported that physical control is achieved through the alteration of the substrata in the snails habitats, causing the death of large numbers and interrupting the propagation of those surviving the new conditions. The concrete lining of canals contributes in controlling snail populations, because higher flow speeds can be maintained and weed growth is minimized and during the periods that irrigation water is not needed, canals can be left to dry, snail populations will diminish and aquatic weeds can be eliminated [**7**].

The aim of the present study is to evaluate the impact of certain lining materials such as cement and plastic as well as mud substratum of the water bodies on the survival and infection rates of *B. alexandrina* snails by *Schistosoma mansoni* miracidia as well as the cercarial production from snails was also determined.

II. Materials and Methods

A. Snails

Experiments were carried out with *B. alexandrina* snails, the intermediate host of *Schistosoma mansoni* in Egypt. Snails were collected from irrigation canals in Giza

Governorate. Parasite free snails had been acclimatized to laboratory conditions. They were maintained in 20 liters of de-chlorinated water in plastic aquaria at a water temperature of 25±2 ºC. They were provided with dried powdered lettuce (*lactuca stativa* L) twice a week. The water was changed every week to avoid bacterial growth and pollution. The 2nd generation of these snails were used as a laboratory ones for the current experiment. [8]-[9]-[10].

B. Schistosoma mansoni Miracidum

S. mansoni miracidia were obtained from Schistosome Biological Supply Centre (SBSC), Theodor Bilharz Research Institute (TBRI) Cairo, Egypt. Miracidia used in the present experiments were freshly emerging (within one hour) and showed active swimming.

C. Mass Infection

Eight experimental aquaria (38.5x32x20 cm) were prepared. Two replicates were used for each lining and control test. The aquaria were designed as: the 1st and 2nd ones provided with mud substratum, the 3rd and 4th ones lined in the bottom and the inside walls with plastic material; 5th and 6th ones lined with cement and the 7th and 8th aquaria remained without lining (as control). Then, groups of laboratory bred *B. alexandrina* snails was added into each aquaria as target snails. Exposure of snails to *S. mansoni* miracidia was carried out in mass (8-10 miracidia/ snail). After 25 days post exposure, snails were individually examined weekly for shedding cercariae. The infection rate (expressed as the number of infected snails divided by the number of survived ones x100), pre-patent period (days), duration of cercarial shedding (days) and the cercariae production for each snail were calculated [11]. The comparison between the different lined materials and those of unlined ones (control test) was recorded.

D. Individual Infection

The same experiment described above in mass infection was done except that the snails were exposed individually to miracidia before introducing into the experimental aquaria. Two levels of miracidia were used in two sets of experiments (low level of 3-5 miracidia/snail & high level of 8-10 miracidia/snail).

III. Results

Results in Table I showed that the mean prepatent period of snails infected with *Schistosoma mansoni* miracidia by mass infection and maintained in aquaria with the substratum mud, cement, plastic and control (without lining) was 22±3.61, 21±0, 28.78±8.17and 21±0, respectively. The mean duration of cercariae shedding was 13.46±6.68, 17.89±7.91, 9.1±6.64 and 15.27±8.17 days for mud, cement, plastic and control respectively. Whereas, Table II showed that the mean pre-patent period of snails exposed to miracidia (8-10 miracidia/snail) by individual infection was 22.17±2.68,21±0, 36.40±3.13 and 23.33±3.42 days for mud, cement, plastic and control, respectively. The mean duration of cercariae shedding was 16.72±8.37, 12.6±3.13, 16.33±5.7, 18.2±6.37 for mud, cement, plastic and control respectively. It was observed that after 4 weeks, snails infected by mass and individual infection then maintained in mud substratum showed a higher significant number of cercarial output (6375 and 3361 cercariae, respectively) compared to those maintained in cement (1617 and 74 cercariae), plastic (1481and1446 cercariae). While, the control groups produced 2088 and 3527 cercariae for mass and individual infection, respectively (Tables I,II & Fig.1). Moreover, the infection rate for snails infected by mass infection and maintained in mud, cement, plastic and control was 81.25%, 75%, 71.43%, 57.89%, respectively. Whereas, this rate was decreased for those infected by individual infection with high level miracidia (78.26%, 50%, 31.58%, 68.18%) and low level (38.88%, 0.0, 30%, 30.77%) in mud, cement, plastic and control, respectively (Tables I,II,III & Fig. 2). A highly significant increase ($p<0.001$) in the mean number of cercariae per snail was produced by snails (mass infection) maintained in mud (334±193.6) compared to those in cement, plastic and control which recorded 61.45±62.11, 78.2±46.2 and 50.35±37.51 cercariae/snail, respectively (Table I). Results in Fig.3 illustrated the number of cercariae/snail/week for snails infected by mass infection. It indicated that snails maintained in mud exhibited two picks at the 2nd and 4th week which represented by 334.71 and 602.5 cercariae/snail, respectively. However, snails maintained in cement showed a higher number of cercariae/snail at the 3rd week (142.71 cercariae/snail) than those in plastic and control (89.0 and 98.7 cercariae/snail, respectively). An extremely highly significant reduction ($p<0.001$) in the mean number of cercariae /snail was observed in snails infected by individual infection (high level) and maintained in cement (4.17±4.9) comparing with those in mud, plastic and control which recorded 75.85±45.29, 78.3±57.48 and 65.1±72.3, respectively (Table II). Results in Fig. 4 showed that the control snails exhibited the highest pick at the 3rd week represented by 171.71 cercariae/snail followed by those snails maintained in plastic and mud which exhibited a pick of 136.2 and 120.19 cercariae/snail, respectively at the 2nd week. In contrast, snails maintained in cement substratum showed the lowest number of cercariae at the 3rd week (9.5 cercariae/ snail). A highly significant increase ($p<0.001$) in the percentage of mortality among snails infected by mass and both high and low individual infection maintained in cement substratum which recorded 40%, 66.7% and 100%, respectively (Tables I,II,III).

TABLE I
EFFECT OF LINING MATERIALS ON THE INFECTION OF *BIOMPHALARIA ALEXANDRINA* SNAILS WITH *SCHISTOSOMA MANSONI* MIRACIDIA (MASS INFECTION:10 MIRACIDIA/SNAIL).

Type of lining	No. of Exposed Snails	Survived snails at the fist cercarial shedding	No of infected snails	Infection rate	Total No of cercariae/snail		Prepatent Period (Days)		Duration of cercarial shedding (Days)		Total No of cercaria production	% of Mortality
					Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD		
Mud	20	16	13	81.25	153.9-602.5	334±193.6	21-35	22±3.6	7-28	13.46±6.7	6375	20
Cement	20	12	9	75.00	6.7-142.7	61.45±62.1	21	21±0	7-28	17.89±7.9	1617	40
Plastic	20	14	10	71.43	18-129.8	78.2±46.2	21-42	28.78±8.2	7-28	9.1±6.6	1481	30
Control	20	19	11	57.89	7.1-98.7	50.35±37.5	21	21±0	7-28	15.27±8..2	2088	5

TABLE II
EFFECT OF LINING MATERIALS ON THE INFECTION OF *BIOMPHALARIA ALEXANDRINA* SNAILS WITH *SCHISTOSOMA MANSONI* MIRACIDIA (HIGH LEVEL: 8-10 MIRACIDIA/SNAIL).

Type of lining	No. of Exposed Snails	Survived snails at the first cercarial shedding	No of infected snails	Infection rate	Total No of cercaria/snail		Prepatent Period (Days)		Duration of cercarial shedding (Days)		Total No of cercaria production	% of Mortality
					Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD		
mud	30	23	18	78.26	26.9 - 120.2	75.85±45.29	21-28	22.17±2.68	7-28	16.72±8.37	3361	23.33
cement	30	10	5	50*	0.0 - 9.5	4.17±4.9***	21	21±0	7-14	12.6±3.13	74***	66.7
Plastic	30	19	6	31.58***	78 - 136.2	78.3±57.48	35-42	36.40±3.13	7-21	16.33±5.7	1446	36.7
control	30	22	15	68.18	14 - 171.7	65.1±72.3	21-28	23.33±3.42	7-28	18.2±6.37	3527	26.7

*** extremely highly significant ($p<0.001$) relative to mud and control groups
* highly significant ($p<0.05$) relative to mud and control gro

TABLE III
EFFECT OF LINING MATERIALS ON THE INFECTION OF *BIOMPHALARIAALEXANDRINA* SNAILS WITH *SCHISTOSOMA MANSONI* MIRACIDIA (LOW LEVEL: 3-5 MIRACIDIA/SNAIL).

Type of lining	No. of Exposed Snails	Survived snails at the first cercarial shedding	No of infected snails	Infection rate	% of Mortality
Mud	30	18	7	38.88	40
Cement	30	0	0	0.00	100
Plastic	30	10	3	30.00	66.7
Control	30	13	4	30.77	56.7

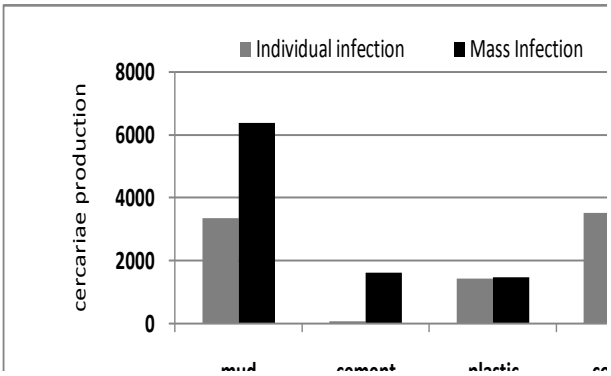

Fig. 1 Total number of cercariae production produced by *Biomphalaria alexandrina* snails maintained in different lining materials by individual infection (8-10 miracidium/snail)&mass infection (10 miracidium/ snail).

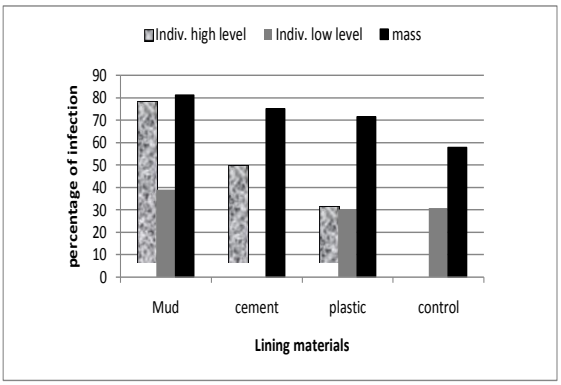

Fig. 2. Effect of lining materials on the infection rate of *Biomphlaria alexandrina* snails by individual infection (high level:8-10 miracidia/snail and low level :3-5 miracidia/snail) and mass infection.

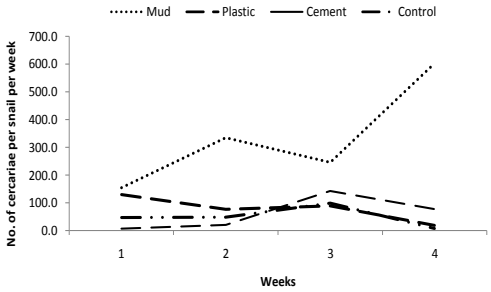

Fig. 3. Number of cercariae/snail /week produced by *Biomphalaria alexandrina* snails maintained in different lining materials by mass infection (10 miracidium/snail).

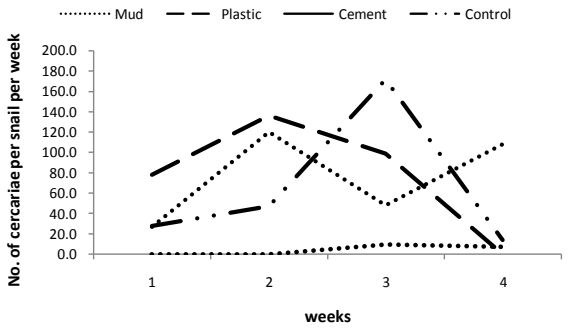

Fig. 4. Number of cercariae/snail /week produced by *Biomphalaria alexandrina* snails maintained in different lining materials by individual infection (8-10 miracidium/ snail).

IV. DISCUSSION

Effect of lining water bodies with cement, plastic and mud on the infection of *B. alexandrina* by *Schistosoma mansoni* miracidia was studied in the present work. Snails infected by both mass and individual infection then maintained in mud substratum produced a higher significant number of cercarial output (6375 and 3361 cercariae, respectively) during four weeks after shedding compared to those maintained in aquaria lined with cement (1617 and 74 cercariae, respectively), plastic (1481and1446 cercariae, respectively). While, the control groups produced 2088 and 3527 cercariae for mass and individual infection, respectively. Thus, in all cases of lining and control groups, it was found that the number of cercariae shedding from snails was the highest in case of mass infection compared to those snails infected individually. Also, present data proved that the highest infection rate was found in snails infected by mass and individual infection and maintained in mud substratum compared to those maintained in cement substratum, plastic and control ones. However, the highly percentage of infected snails maintained in cement and plastic (infected by mass infection) comparing with the control group was attributed and correlated with the highly percentage of mortality among snails in cement and plastic substratum (40% and 30% respectively) whereas, it was only 5% among control snails. It is suggested that miracidia were enter the snails escaping from the stress of lining materials, but when infected snails, it produced a lower number of cercariae compared to those maintained in mud and control ones. Thus, although the high level of infection rate among snails in cement and plastic, the cercariae out put was lower than the control snails. Present results indicated that a highly significant increase in the mean number of cercariae per snail was produced by snails (mass infection) maintained in mud substratum compared to those in cement, plastic and control. An extremely highly reduction in the mean number of cercariae/snail was observed in snails infected by individual infection and maintained in cement substratum comparing with those in plastic, mud and control. This may be due to the highly significant increase in the mortality rate among snails maintained in aquaria lined by cement in which the number of infected snails was only five compared to those maintained in mud and control ones. Although the highest mean number of cercariae/snail was appeared in case of snails maintained in aquaria lined with plastic, it was observed that the number of infected snails (only 6 snails) was very lower than those maintained in mud and control ones (18 and 15 snails, respectively) and this explain the lowest total number of cercariae production from snails in plastic than those in mud and control. The present results go well with those of [12] who stated that for

minimizing the risk of infection from new water conservation, irrigation schemes is by lining canals with cement and keeping them free from silt and vegetation in which snails can breed. Similarly, reference [13] reported that each environmental change, whether occurring as a natural phenomenon or through human intervention, changes the ecological balance and context within which disease hosts or vectors and parasites breed, develop, and transmit disease. Each species occupies a particular ecological niche and vector species sub-populations are distinct behaviourally and genetically as they adapt to man-made environments. Also, present results about the lower number of cerrcariae out put from snails maintained in cement and plastic substratum may reflect and contributed to the data obtained by [14] who used the electrophoratic (SDS-PAGE) pattern of tissue soluble proteins extracted from *B. alexandrina* snails. The highest number of protein bands were appeared in extracted tissue soluble proteins of *B. alexandrina* snails maintained in cement substratum (18 bands) after 14 days with the appearance of additional bands (molecular weight: 6.81KDa). They suggested that these are stress bands appeared only in snails maintained in cement and may be explain the higher percentage of snails mortality after exposure to cement lining. They added that the lowest number of bands (13 bands) appeared in extracted tissue soluble proteins of *B. alexandrina* snails maintained in plastic substratum after 7days exposure. Moreover, the maximal molecular weight (186.84KDa) was appeared in proteins extracted of *B. alexandrina* snails maintained in cement substratum. Similarly, reference [15] used the same technique to separate tissue protein of control and trematode infected *B. alexandrina* snails. The authors discussed the correlation between separation pattern and metabolic redirection of the snail host by the developing sporocysts. The electrophoretic analysis of tissue soluble proteins has been used by [16] to determine the relationships between ova of the parasites *Schistosoma mansoni, Echinostoma liei, Schistosoma haematobium and Fasciola gigantica* and their snail hosts *B. alexandrina, B. glabrata ,Bulinus truncatus and Lymnaea natalensis,* respectively. The results revealed that similar protein bands were found in the parasites and their hosts.

Thus, the present investigations on the effect of lining water courses with cement or plastic materials on the susceptibility of *B. alexandrina* snails to *S. mansoni* infection are of great importance in epidemiological studies and control of schistosomiasis.

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