Journal of Bacteriology and Parasitology

Mohamed et al. J Bacteriol Parasitol 2011, 2:1
DOI: 10.4172/2155-9597.1000104

Research Article Open Access

Tissue Responses Exhibited by *Biomphalaria Alexandrina* Snails from Different Egyptian Localities Following Exposure to *Schistosoma Mansoni* Miracidia

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Abstract

Snails' susceptibilities to infection with *Schistosoma mansoni* were determined through observation of infection rates, total cercarial production and tissue responses of the first generation (F_1) of *Biomphalaria alexandrina* snails originally collected from different Egyptian governorates (Giza, Fayoum, Kafr El-Sheikh, Ismailia and Damietta). *B. alexandrina* snails from Schistosome Biological Supply Center (SBSC, TBRI), Giza, Egypt were used as a reference control group. *S. mansoni* miracidia from SBSC were used for snail infection. Snails' responses towards penetrating *S. mansoni* miracidia were compared between groups. The emergence of cercariae for a three-month period and the calculation of survival and infection rates, in control (Schistosome Biological Supply Center-SBSC) and infected snails were evaluated. The results indicated SBSC and Giza snails showed a greater susceptibility to infection and lower mortality rates. In addition, at 6 and 72 hrs post-exposure to miracidia all the snail groups showed no difference in the anatomical locations of sporocysts. The larvae were found in the head-foot, the mantle collar and the tentacles of the snails. Sporocysts showed normal development with low tissue reactions in SBSC and Giza snail groups infected with *S. mansoni* miracidia (Giza origin). However, in Fayoum, Kafr El-Sheikh, Ismailia and Damietta snail groups, variable tissue responses were observed in which numerous hemocytes made direct contact with *S. mansoni* larvae forming capsules. The results suggested that, different responses of *B. alexandrina* snail's hemocytes towards *S. mansoni* are related to the degree of susceptibility of these snails. So this is important in planning the strategy of schistosomiasis control.

Keywords: *Biomphalaria alexandrina*; *Schistosoma mansoni*; Resistance; Susceptibility; Hemocytes; Encapsulation

Introduction

The host-parasite relationship is complex and questions remain concerning the susceptibility of snails to infection by the respective trematodes and their suitability as hosts for continued parasite development. The dynamic interaction between molluscs and their trematode parasites leads either to a state of co-existence, in which the trematode thrives and produces subsequent stages of its life-cycle, or to incompatibility, where the trematode is either destroyed and eliminated by the host snail defensive responses or fails to develop because the host is physiologically unsuitable [1,2]. Successful colonization of a compatible snail host by a digenetic trematode miracidium initiates a complex proliferative development program requiring weeks to reach culmination in the form of production of cercariae which, once started, may persist for the remainder of the life span of the infected snail [3].

Geographical distribution of intestinal schistosomiasis is directly associated with the presence of susceptible snails of the genus *Biomphalaria* and the etiological agent, *S. mansoni*. This trematode is a stenoxenic parasite, i.e., it uses specific intermediate host species [4]. However, not all *Biomphalaria* species are susceptible to *S. mansoni*. *Biomphalaria* susceptibility to *S. mansoni* infection varies among snails according to different ages, genetic variation, immune system status and geographic areas in which both snails and the trematode occur [5].

During the life cycle of *S. mansoni*, sporocysts larval stages develop in the mollusc intermediate hosts. Parasites need to penetrate into this host, develop, multiply asexually and finally leave the host to continue their life cycle [6,7]. Parasites therefore face many challenges such as gaining enough energy to grow and to evade the defense system of the host [8,9]. In parallel, hosts have to co-evolve with their parasites to avoid being infected. Susceptibility or resistance to infection in planorbid snails by *S. mansoni* is regulated genetically in a way that some susceptibility may be present in resistant snails [10,11].

Many studies have been done to investigate the mechanisms by which the snail resistance is achieved [12,13]. From these studies, immune response of the snail intermediate host *B. glabrata* is determined through complex relationship involving circulating hemocytes and the early larval stages of the parasite. In resistant snails, hemocytes recognize and destroy the parasite via a cellular encapsulation response that may involve plasma activating or recognition factors, lysosomal enzymes or other cytotoxic elements and phagocytosis of the damaged parasite tegument. Susceptibility generally is viewed to be the result of hemocytes failure to recognize and/or mount an effective cytotoxic response against the invasive parasite larvae. Mohamed et al. [14] reported that the natural intermediate host of *S. mansoni* in Egypt is refractory to infection with the Puerto Rican strain of this parasite. Although the miracidia of the parasite successfully penetrate the snail,

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Received September 06, 2010; **Accepted** October 28, 2010; **Published** October 30, 2010

Citation: Mohamed AH, El-Din ATS, Mohamed AM, Habib MR (2010) Tissue Responses Exhibited by *Biomphalaria Alexandrina* Snails from Different Egyptian Localities Following Exposure to *Schistosoma Mansoni* Miracidia. J Bacteriol Parasitol 2:104. doi:10.4172/2155-9597.1000104

yet they are quickly subjected to strong tissue reactions leading into the encapsulation of the parasite larvae followed by degeneration and eventual exclusion from the snails' tissues.

Populations of snails of the same species show different degrees of susceptibility to infection [15]. Loker and Bayne [16] reported that the great majority of sporocysts incubated in the plasma of susceptible snails and later put into contact with amoebocytes originating from resistant snails were destroyed. When the sporocysts were incubated in plasma from resistant snails and later exposed to amoebocytes of susceptible snails, no destruction of the larvae was noted. Souza et al. [17] made a comparative study of the development of *S. mansoni* during the intramolluscan phase by mean of histological sections of *B. tenagophila, B. straminea* and *B. glabrata* from Brazil; they did not found larvae in snails fixed 72 hrs after exposure. In specimens shedding cercariae, 31 days after exposure tissue reactions encapsulating the larvae were seen in *B. tenagophila* and *B. straminea*, in the head-foot, mantle collar and renal ducts explaining the lower levels of infection and average numbers of cercariae shed by these two species.

The purpose of this study is analyze susceptibility [infection rate] and detect the differences in hemocytes reactions against the penetrated *S. mansoni* parasite in tissues of *B. alexandrina* snails collected from different Egyptian governorates.

Materials and Methods

Snails

The snails used were laboratory-bred *B. alexandrina* snails (F_1) originated from Five snail groups collected from Egyptian governorates (Giza, Fayoum, Kafr El-Sheikh, Ismailia and Damietta). In addition, a sixth group of *B. alexandrina* snails were used as reference control obtained from Schistosome Biological Supply Center, Theodor Bilharz Research Institute, Giza, Egypt (SBSC-TBRI).

S. mansoni

S. mansoni ova were obtained from SBSC-TBRI which was originally an Egyptian strain obtained from Giza Governorate and has been routinely maintained in *B. alexandrina* and albino mice *Mus musculus* CD1 strain. They were left for hatching in dechlorinated water (24±1°C) under a desk lamp. The hatched miracidia were pipitted for snail infection [18].

Snail exposure to miracidia

Three replicates, each of 30 lab-bred *B. alexandrina* snails (4-6 mm), from each governorate offspring were individually exposed to ten newly hatched *S. mansoni* miracidia (SBSC) suctioned by micropipette according to Theron et al. [19]. Examination of exposed snails for cercarial shedding.

Starting from the day 21 post miracidial exposure, the snails were examined individually and repeatedly for cercarial shedding in multi dishes under artificial light for two hours (stimulant period) and 2 ml of dechlorinated tape water/snail. After initial shedding was observed, snails were screened individually once weekly till the death of snails. All snails that died during the prepatent period were crushed between two slides and inspected under a microscope for immature parasite stages. The snail's infection rate was calculated at the end of experiment by dividing number of shedding and positive crushed snails on the number of exposed snails and the survival rate was calculated by dividing the number of snails at first shedding on the total number of exposed snails Yousif *et al.* [20]. At the first day of detecting cercariae, positive snails were separated individually in a plastic cups. The produced cercariae/snail were transferred to a small Petri dish by a Pasteur pipette, fixed in Bouin's solution and counted under a stereomicroscope. This examination was repeated weekly.

Histological investigations

At intervals of six and seventy-two hours post miracidial exposure, five snails from each snail group were carefully crushed between two glass slides, the shell fragments were removed under a dissecting microscope. Head-foots regions were separated and immediately fixed in alcoholic Bouin's fluid {(15 ml picric acid (saturated aqueous solution), 5 ml of 40% formalin, and 1 ml of 100% glacial acetic acid)} for 12 hours. After fixation, specimens were dehydrated in an ascending series of alcohol (70%, 80%, 90% and 100%) each 15 minutes. The specimens were cleared in two changes of xylene and embedded in molten paraplast at 60°C. Serial sections were cut at 5µ thickness using rotary microtome and stained with Ehrlich's haematoxylin and counterstained eosin [21]. The sections were then mounted by DPX and covered by glass cover. Histological sections were examined and photographed with automatic camera using Olympus System Microscope BX2 Series [BX41, Japan] to detect any hemocytes reactions against the parasite.

Statistical analysis

Data for the number of cercariae produced were square root transformed before statistical analyses to satisfy the distributional assumptions of the test. ANOVA were performed according to Sokal and Rohlf [22] on cercarial shedding with week as a repetition factor and parasitic infection as the treatment factor. Experimental infection rates of every two snail groups were compared by means of 2 x 2 contingency tables, using the chi-square (χ^2) test. Significant differences were considered at $p \leq 0.05$. Data were expressed as mean ± standard error of the mean (SEM).

Results

Snail's survival rate at first shedding

The survival rate of different snail groups exposed to *S. mansoni* miracidia [SBSC strain], at first cercarial shedding, was highest in Giza group [87%], while the lowest one was recorded in Ismailia group being 40%. In between there were variable percentages for the other snail groups; 85% for Fayoum, 60% for SBSC, 50% for Kafr El-Sheikh and 43% for Damietta snail groups (Figure 1). The differences observed in the survival rates among the five snail groups were significantly increased in Giza and Fayoum snail groups, while it was significantly decreased in Ismailia and Damietta groups compared to SBSC snail group.

Snail's infection rate

The highest infection rate among the six *S. mansoni* exposed *B. alexandrina* snail groups was that of SBSC group with infection rate 50.3%. On the other hand, *B. alexandrina* snail groups from Ismailia and Kafr El-Sheikh showed an equal low infection rate (20%), while a moderately high percent (33.3%) was obtained with Damietta group (Figure 1). The differences in the infection rates of the five snail groups compared to that of SBSC group, were decreased significantly ($p<0.001$) in all groups except in Giza group (43.3%) which showed no significant difference.

Mean total number of cercariae per snails

A marked variation in the general crecarial outputs was observed among the six *B. alexandrina* snail groups. The highest mean total

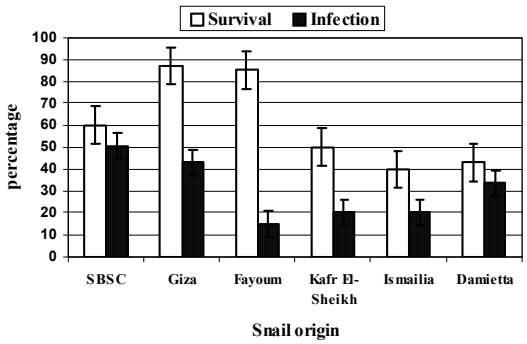

Figure 1: The survival and infection rates of six *Biomphalaria alexandrina* snail groups from SBSC and five Egyptian governorates infected with *Schistosoma mansoni* miracidia from SBSC. P< 0.05 significant differences compared to *B. alexandrina* reference control (SBSC-TBRI strain) Data was expressed as Mean ± SD.

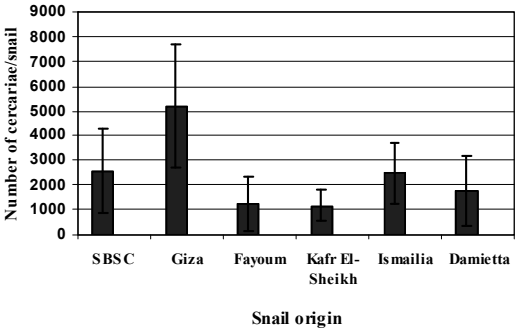

Figure 2: Mean total number of cercariae per snail for *Biomphalaria alexandrina* snail groups from SBSC and five Egyptian governorates infected with *Schistosoma mansoni* miracidia from SBSC.

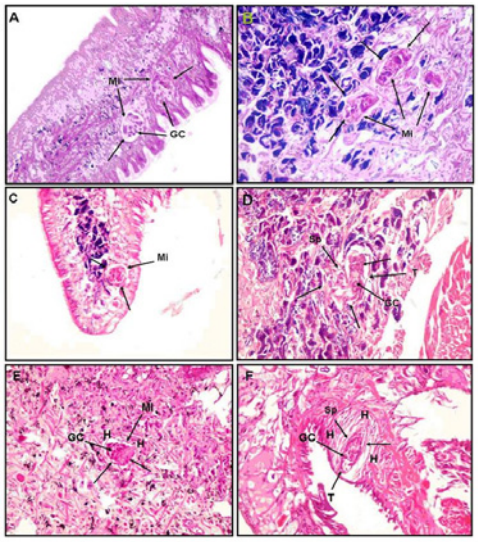

Figure 3: T.S of *Biomphalaria alexandrina* snails exposed to *Schistosoma mansoni* miracidia: left panel (6hr post exposure), right panel (72hr post exposure). A&B: snails from SBSC, C&D: Giza snails, A&C: the normal miracidium "Mi" (arrows) in tentacle and mantle; note absence of hemocytes response, B&D: normally developing elongated sporocyst "Sp" (arrows) in head-foot region. E&F: Fayoum snails. E: miracidium "Mi" (arrows) in head-foot region surrounded by few hemocytes "H", F: active infiltration around the sporocyst "Sp" which is surrounded by several layers of flattened hemocytes "H" in the head-foot region at the basement of the tentacle germinal cell (GC); tegument (T) (h & e) (x 400).

number of cercariae per snail was 5198.17 ± 2486.1 in Giza group, while the lowest mean total number of cercariae per snail was 1175.33 ± 626.99 in Kafr El-Sheikh group. The mean number of crecariae per snail was highly significant in Giza and Kafr El-Sheikh snail groups ($p<0.01$) compared to SBSC-TBRI *B. alexandrina* group (Figure 2).

Histological observations

The present results showed that, at each time post miracidial exposure (6 and 72 hrs), all *B. alexandrina* snail groups showed no difference in the anatomical locations of miracidia and sporocysts, the larvae were found in the head-foot, the mantle collar and the tentacles of the snails.

Six-hours post exposure, some miracidia developed apparently normally, while others underwent encapsulation, the penetrating miracidia were surrounded by numerous hemocytes in snails originated from Fayoum (Figure 2 E), Ismailia (Figure 4 A), Kafr El-Sheikh (Figure 3 C) and Damietta (Figure 4 E). While no cellular reactions was usually observed and miracidia showed normal development in snails of SBSC (Figure 3 A) and snails from Giza (Figure 3 C). These two snail groups showed a lower hemocytes response to penetrating miracidia. Some miracidia had already induced migration of hemocytes to their vicinity as in Damietta snails although of its moderate susceptibility to *S. mansoni* infection (Figure 4 E).

Seventy two-hours post *S. mansoni* exposure, mother sporocysts were observed in various stages of developmental or deterioration in tissue sections of the different *B. alexandrina* snail groups investigated. In snails obtained from SBSC and Giza, *S. mansoni* mother sporocysts showed normal development, most germinal cells stained normally had characteristic nucleoli which seemed to be proliferate. There was no contact of the sporocysts' surface with hemocytes. No host cellular response was usually observed around sporocysts. Sporocysts had elongated into a thin-walled sac with transverse constrictions and contained proliferating germinal tissue (Figure 3 B and D).

In snails originated from Fayoum, Ismailia, Kafr El-Sheikh and Damietta, a host cellular reaction was observed around the sporocysts. Hemocytes had made direct contact with the sporocysts and usually formed capsules. Capsules were spherical, as seen in Damietta snails (Figure 4 F), or oval shape (Kafr El-Sheikh snails- Figure 4 D).

Discussion

The present results showed clear differences in the degree of susceptibility of snail populations originating from some localities in Egypt to infection with *S. mansoni* strain from SBSC-TBRI. *B. alexandrina* from SBSC and Giza exhibited the highest degrees of susceptibility amongst snail populations studied during the present investigation. The infection rates were 50.3% and 43.3%, respectively. These variations in susceptibility agrees with Farndsen [23] who recorded that *B. alexandrina* snails from various localities showed different susceptibility rates to a specific strain of *S. mansoni.* In the same context, Bakry [24] reported that *B. alexandrina* snails from Damietta were less susceptible to infection with an Egyptian strain of *S. mansoni* [Giza] than *B .alexandrina* from Fayoum and Giza.

The highest infection rate exhibited by the snails of SBSC (50.3%) and Giza (43.3%) reflect higher susceptibility to schistosome infection, since the source of both snail and parasite considered the same. This is agreeing with the theory of local adaptation of the parasite to its snail host [25,26].

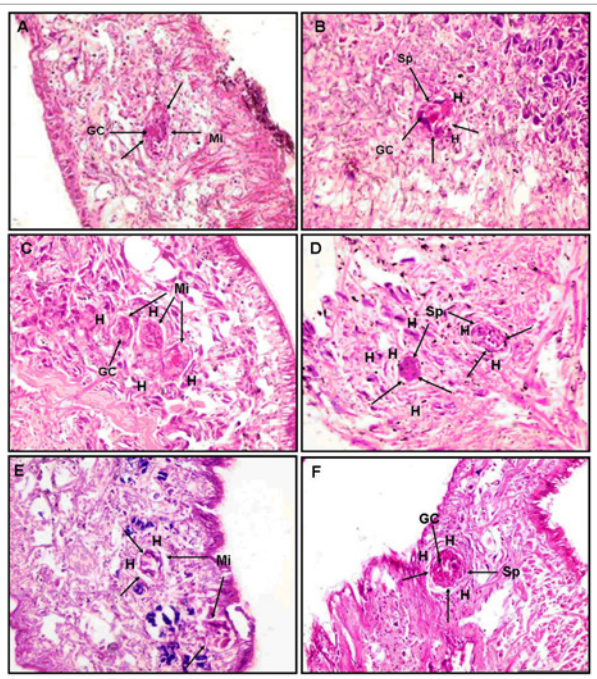

Figure 4: T.S of *Biomphalaria alexandrina* snails exposed to *Schistosoma mansoni* miracidia (SBSC): left panel (6hr post exposure), right panel (72hr post exposure). A&B: Ismailia snails, A: miracidium "Mi" (arrows) in head-foot region; note successful penetration of miracidia without tissue response at this time, B: hemocytes "H" infiltration around the abnormally developed sporocyst "Sp" in the head-foot region as an inward step of forming a capsule, C&D: Kafr El-Sheikh snails, C: three encapsulated miracidia "Mi" (arrows) in head-foot region; note obvious tissue reactions through hemocytes "H" aggregations around the miracidia, D: hemocytes "H" infiltration around the abnormally developed sporocysts "Sp" in the head-foot region forming a capsule, E&F: Damietta snails, E: two miracidia "Mi" (arrows) in tentacle; Note haemocytic response "H", F: degraded sporocyst "Sp" and large capsule formation by several layers of hemocytes "H" in the head-foot region near the tentacle; germinal cell (GC); tegument (T) (h & e) (x 400).

The present results indicated that the first generation (F_1) of different *B. alexandrina* snail groups collected from different geographic areas in Egypt acquired infection with *S. mansoni* but the snails exhibited different histological responses towards penetrating *S. mansoni* parasite. This is in accordance with Théron et al. [19] who demonstrated that for the same species/species host-parasite couple the intraspecific differences occurs between two geographical combinations. In the present study, different cellular responses were observed in *B alexandrina* snails of Fayoum, Ismailia and Kafr El-Sheikh (low susceptible) and even in moderately susceptible snails from Damietta.

Miracidia and mother sporocysts were found in the head-foot, tentacles and mantle collar in all *B. alexandrina* groups after 6 hrs and 72 hrs of exposure to *S. mansoni* miracidia (SBSC). This is in accordance with the majority of previous observations on *Biomphalaria* snails infected with *S. mansoni* [8,27-29]. However, Théron et al. [19] demonstrated experimentally that, distribution patterns of schistosome larvae among the snail host population may differ depending upon the host–parasite combination.

Seventy two-hours post *S. mansoni* miracidial exposure, sporocysts were observed in various stages of developmental or deterioration in tissue sections of the different *B. alexandrina* groups investigated. With compatible *B. alexandrina* snail hosts obtained from SBSC and Giza, *S. mansoni* mother sporocysts showed a normal development following the usual scheme mentioned by Pan [27] and there was no contact of the sporocysts surface with hemocytes. In the same context, Théron and Coustau [30] stated that in natural populations some snail/schistosome combinations are compatible and others are not. In compatible interactions, the parasite penetrates and develops normally within the snail, giving rise to the next parasite stage, the cercariae. Alternatively, in incompatible interactions, the larval trematode penetrates but is immediately recognized as non-self, encapsulated and destroyed by the mollusc's internal defense system.

The sporocysts had elongated into a thin-walled sac with transverse constrictions and contained proliferating germinal tissue in SBSC and Giza snails. However, in snails originating from Fayoum, Ismailia and Kafr El-Sheikh a host cellular reaction was observed around the sporocysts. Hemocytes had made direct contact with the sporocysts which had not increased considerably [no elongation occurred] this means that development of the mother sporocysts may be stopped after approximately 24 hrs. This agrees with Loker et al. [31] who found that during infection with the parasite *S. mansoni*, hemocytes of resistant *B. glabrata* snails execute a rapid defense, encapsulating the parasite in less than 24 hrs, and ultimately destroying it [32].

A lack of response of hemocytes towards the parasite is also characteristic for the compatible systems represented by the susceptible *B. alexandrina* stocks of SBSC-TBRI and Giza when infected with *S. mansoni* of SBSC-TBRI. These two snail groups showed more susceptibility and higher cercarial output than the other snail groups. This agrees with Newton [33] who stated that susceptible snails give rise to variable numbers of cercariae and those which are very susceptible can shed numerous cercariae, with no overt reactions, their tissues appearing tolerant to the presence of the multiplying and growing sporocysts. In this respect, McLaren and Terry [34] reported that in *B. glabrata* snails susceptible to *S. mansoni*, the parasites might interfere with the ability of hemocytes to encapsulate and destroy them; sporocysts might evade the snails' defense system by antigenic mimicry, whereby the parasite expresses surface antigens that cross-react with self (snail) molecules. Also, Philips et al. [35] suggested that the plasma of susceptible snails might contain factors that allow the parasite to evade snail defenses. In the present study, snails originating from Damietta were considered moderately susceptible to infection with *S. mansoni*, however, miracidia induced migration of hemocytes to their vicinity and some of them were surrounded by numerous hemocytes (encapsulation).

Adema et al. [36] stated that the immune defenses of *B. glabrata* distinguish and respond differently to various immune challenges. Many investigators observed cellular reaction against trematode invasion such reactions usually consist of massive proliferation of amebocytes, with encapsulation and destruction of sporocysts [27,37]. Similarly Loker et al. [38] found miracidium-amebocyte contact within 3 hrs and phagocytosis of sporocysts microvilli and pieces of tegument within 7.5 hrs, while extensive pathology was demonstrated within 24 hrs and by 48 hrs only scattered remnants of sporocysts remained. Hemocytes contact with sporocysts is essentials for rapid sporocysts death *in vivo* and most sporocysts of *S. mansoni* were dead within 72 hrs [39].

In the present study, typical capsules were observed 72 hrs post miracidial exposure and a number of up to four layers of accumulating hemocytes surrounded the mother sporocysts. These multiple layers of hemocytes act as a wall that isolates the sporocyst preventing the uptake of nutrients present in the hemolymph of the snails [40]. Such

hemocytes responses have been described and reported especially in snails resistant to dignean trematodes in light and electron microscopic as well as in *in vivo* and *in vitro* studies [31,41]. Moreover, Guaraldo et al. [42] and Hahn et al. [43] studied the reactions of tissues in *B. glabrata* and *B. tenagophila* from the first hours until the eighth week following infection and observed that there was slight amoebocitary reaction around the sporocysts in *B. glabrata*, whereas there was a strong reaction of the tissues in *B. tenagophila*. As stated by many authors [44,45] that the snails´ defense generally occurs by means of destruction, total or partial, of the primary sporocyst at the first few hours following the penetration of the miracidium. The results also confirm that the hemocytes could be the effector element in the destruction mechanism of trematodes, being directly involved in the death of some encapsulated parasites [2,45].

In conclusion, The offspring (F_1) of collected *B. alexandrina* snails from different geographic areas in Egypt exhibited different histological responses towards penetrating *S. mansoni* parasite and a very low response of snail hemocytes towards the parasite is characteristic for the susceptible *B. alexandrina* stocks from SBSC and Giza. This is important for understanding the epidemiology of schistosome parasite among natural populations of snails, as well in the decision of schistosomiasis control programs.

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