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Tissue Responses Exhibited by *Biomphalaria alexandrina* Snails from Different Egyptian Localities Following *Schistosoma mansoni* Exposure

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Abstract: Snail's 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 *B. alexandrina* snails originally collected from different Egyptian governorates (Giza, Fayoum, Kafr El-Sheikh, Ismailia and Damietta) were studied comparatively. The elimination 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. SBSC and Giza snails showed greater susceptibilities to infection and lower mortality rates. The results indicated that at 6 and 72 hrs post exposure 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 SBSC and Giza snail groups and *S. mansoni* (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 and usually formed capsules around. 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.

Key words: *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 mollusc 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 schistosomiasis *mansoni* 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

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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 [12-14]. 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 element 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.* [15] reported that the natural intermediate host of *S. mansoni* in Egypt is completely resistant [incompatible case] to infection with the peurto Rican snail group of this parasite. Although the miracidia of the parasite successfully penetrate the snail, 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 snail tissues.

Populations of snails of the same species show different degrees of susceptibility to infection [16]. Loker and Bayne [17] 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.* [18] 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 found that there were not any observed larvae in snails fixed 72 hr 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.

MATERALS AND METHODS

Snails: The snails used were laboratory-bred *B. alexandrina* snails (F_1) originated from Five Egyptian governorates [Giza, Fayoum, Kafr El-Sheikh, Ismailia and Damietta]. Besides, sixth group of *B. alexandrina* snails was 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.

Snail Exposure to Miracidia: Three replicates, each of 30 lab-bred *B. alexandrina* snails [4-6 mm in diameter], from each governorate offspring were individually exposed to ten newly hatched *S. mansoni* miracidia (SBSC) suctioned by micropipette according to Theron *et al.* [19]. Starting from the day 21 post miracidial exposure, the snails were examined individually and repeatedly for cercarial shedding. The snail's infection rate was calculated according to Yousif *et al.* [20].

Histological Investigations: At time intervals 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 using pointed forceps under a dissecting microscope. Head-foots 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 5 μ 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.

RESULTS AND DISCUSSION

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

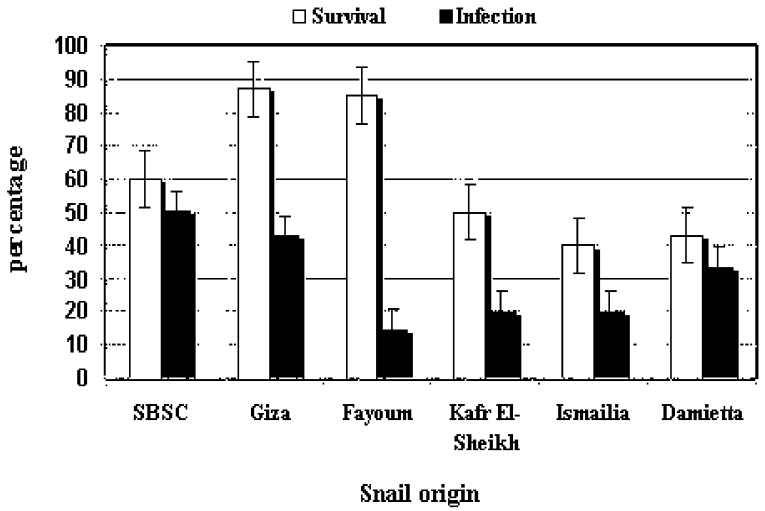

Fig. 1: The survival and infection rates of six *Biomphalaria alexandrina* groups from SBSC and five Egyptian governorates infected with *Schistosoma mansoni* from SBSC

Giza group [87%], while the lowest one was recorded in Ismailia group being 40%. In between these two values there were variable percentages of survival rates recorded for the other snail groups; 85% (Fayoum), 60% (SBSC), 50% (Kafr El-Sheikh) and 43% for Damietta snail group (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.

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 infection rate (20%), while a moderately higher 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.

Total Cercarial Production: A marked variation in the general crecarial outputs was observed among the six snail groups. The maximum total number of crcarial production was obtained from Giza snails being 62,378 cercariae while the lowest number of shedding cercariae (5,070 cercariae) was recorded for Fayoum snails. There was some degree of proximity between the total cercarial production of Ismailia (14,971 cercariae) and Damietta groups (17,448 cercariae).

Histological Observations: The present results showed that, at each time post exposure (6 and 72 hr), 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 3 A), Kafr El-Sheikh (Figure 3 C) and Damietta (Figure 3 E). While no cellular reactions was usually observed and miracidia showed normal development in snails of SBSC [Figure 2 A] and snails from Giza [Figure 2 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 3 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 2 B and D).

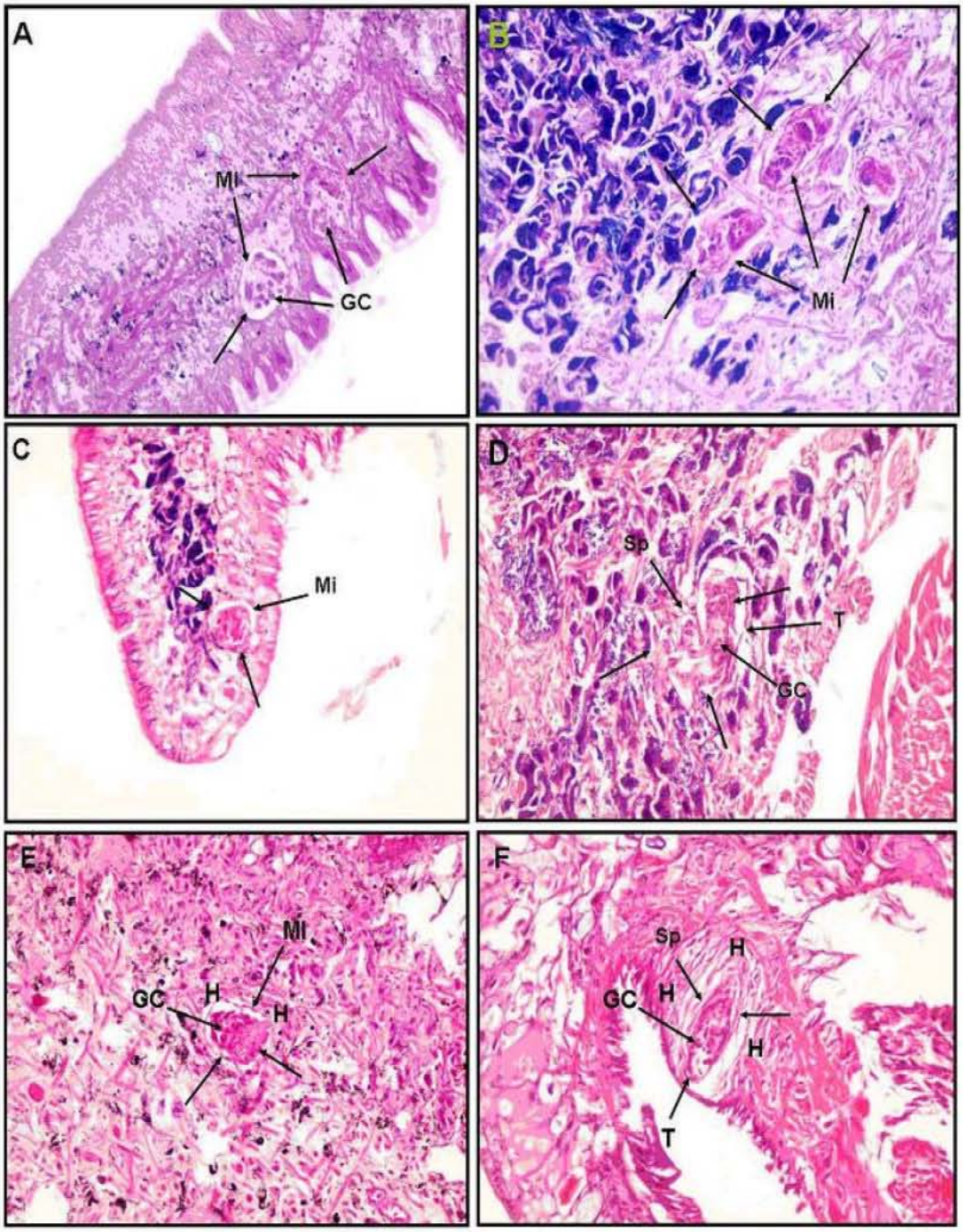

Fig. 2: T.S of *Biomphalaria alexandrina* snails exposed to *Schistosoma mansoni* infection: left panel (6hr post exposure), right panel (72hr post exposure). Notice in A and B (snails from SBSC) C and D (Giza snails), A and C: the normal miracidium "Mi" (arrows) in tentacle and mantle; note absence of hemocytes response. In B and D, normally developing elongated sporocyst "Sp" (arrows) in head-foot. E and F (Fayoum snails). Notice in E: miracidium "Mi" (arrows) in head-foot surrounded by few hemocytes "H". In F: showing 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 and e) (x 400).

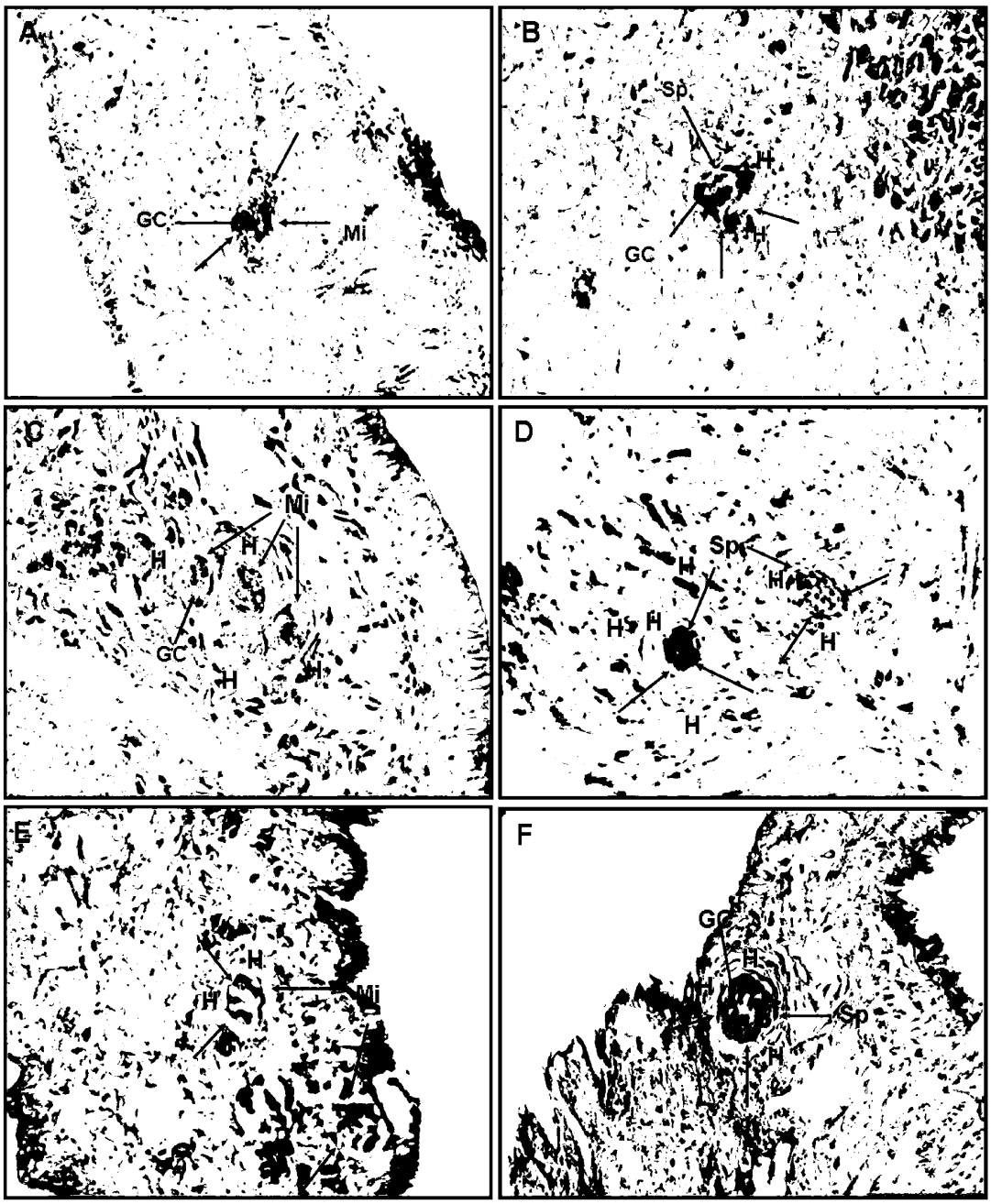

Fig. 3: T.S of *Biomphalaria alexandrina* snails exposed to *Schistosoma mansoni* infection (SBSC): left panel (6hr post exposure), right panel (72hr post exposure). Notice in A and B (Ismailia snails). In A: miracidium "Mi" (arrows) in head-foot; note successful penetration of miracidia without tissue response at this time interval. In B: hemocytes "H" infiltration around the abnormally developed sporocyst "Sp" in the head-foot region as an inward step of forming a capsule. C and D (Kafr El-Sheikh snails). Notice in C: three encapsulated miracidia "Mi" (arrows) in head-foot; note obvious tissue reactions through hemocytes "H" aggregations around the miracidia. In D: hemocytes "H" infiltration around the abnormally developed sporocysts "Sp" in the head-foot region forming a capsule. E and F (Damietta snails). Notice in E: two miracidia "Mi" (arrows) in tentacle; Note haemocytic response "H". In F: degraded sporocyst "Sp" and large capsule formation by several layers of hemocytes "H" in the head-foot near the tentacle; germinal cell (GC); tegument (T) (h and e) (x 400).

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 3 F), or oval shape (Kafr El-Sheikh snails-Figure 3 D).

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 [22] who recorded that *B. alexandrina* snails from various localities showed different susceptibility rates to a strain of *S. mansoni.* In the same context, Bakry [23] 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 [24,25].

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 occur 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 hr and 72 hr of exposure to *S. mansoni* (SBSC). This is in accordance with the majority of previous observations on *Biomphalaria* snails infected with *S. mansoni* [8,26-28]. 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* 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 [26] and there was no contact of the sporocysts surface with hemocytes. In the same context, Théron and Coustau [29] 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. While, in snails originated 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 hours. This agrees with Loker *et al.* [30] 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 hr and ultimately destroying it [31].

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 [32] 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 [33] 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.* [34] 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.* [35] 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 [26,36]. Similarly Loker *et al.* [37] found miracidium-amebocyte contact within 3 hr and phagocytosis of sporocysts microvilli and pieces of tegument within 7.5 hr, while extensive pathology was demonstrated within 24 hr and by 48 hr 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 hr [38].

In the present study, typical capsules were observed 72 hr 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 [39]. 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 [30,40]. Moreover, Guaraldo *et al.* [41] and Hahn *et al.* [42] 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 [43,44] 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 [2,43]

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's hemocytes towards the parasite is characteristic for the susceptible *B. alexandrina* stocks from SBSC and Giza.

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