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

**A.H. Mohamed1, A.T. Sharaf El-Din2*, A.M. Mohamed2 and M.R. Habib2**

1 Zoology Department, Faculty of Science, Menoufia University.
2 Medical Malacology Lab., Theodor Bilharz Research Institute, P.O. Box 30, Imbaba Egypt

**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 (F1) 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 larvae 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, lysozyme 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,

*Corresponding author: A.T. Sharaf El-Din, Department of Environmental Researches and Medical Malacology, Theodor Bilharz Research Institute. P.O. Box 30, Imbaba Egypt, Tel: +2 0107981467, Fax: +2 35408125, E-mail: ahmadsharafeldin@yahoo.com*

**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

**Copyright:** © 2010 Mohamed AH, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
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 find 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 (F₁) originated from five snail groups collected from different 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 cercarial shedding.

**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 tap 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-feet 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 haematoylin 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 (χ²) test. Significant differences were considered at p ≤ 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 cercarial outputs was observed among the six *B. alexandrina* snail groups. The highest mean total
Mean total number of cercariae per snail for *B. alexandrina* miracidia from SBSC. T.S of snails exposed to snails from various localities showed

**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 Farnsden [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].
The present results indicated that the first generation (F1) 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 Kaf 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 Coustdau [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 mollusk'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 Kaf 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, Phillips 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 digenean 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 (F1) 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 schistosomiasis parasite among natural populations of snails, as well in the decision of schistosomiasis control programs.

References

1. Bayne CJ, Yashino TP (1989) Determination of compatibility in mollusca-trematode parasitism. Amer Zoologist 29: 399-406.
2. Van der Knaap WP, Loker ES (1990) Immune mechanisms in trematode-snail interactions. Parasitol Today 6: 175-182.
3. Hanington PC, Lun C, Adema CM, Loker ES (2010) Time series analysis of the transcriptional responses of Biomphalaria glabrata throughout the course of intramolluscan development of Schistosoma mansoni and Echinostoma paraensei. Int J Parasitol 40: 819-831.
4. Caldeira RL, Jannotti-Passos LK, Carvalho OS (2009) Molecular epidemiology of Brazilian Biomphalaria a review of the identification of species and the detection of infected snails. Acta Trop 111: 165.
5. Richards CS, Shade PC (1987) The genetic variation in Biomphalaria glabrata and Schistosoma mansoni. J Parasitol 3: 1146-1151.
6. Davies SJ, Mckenrow JH (2003) Developmental plasticity in schistosomes and other helminths. Int J Parasitol53: 1277-1284.
7. Parker GA, Chubb JC, Ball MA, Roberts GN (2003) Evolution of complex lifecycle in helminth parasites. Nature 425: 480-484.
8. Meuleman EA, Bayne CJ, Van Der Knaap WP (1987) Immunological aspects of snail-trematode interactions. Prog Clin Biol Res 233: 113-127.
9. Dejong-Brink M, Bergamin-Sassen M, Solis Soto M (2001) Multiple strategies of schistosomes to meet their requirements in the intermediate snail host. Parasitology 123: 129-141.
10. Richards CS, Merritt JW (1972) Genetic factors in the susceptibility for juvenile Biomphalaria glabrata to Schistosoma mansoni infection. Amer J Trop Med Hyg 21: 425-434.
11. Carton Y, Nappi AJ, Poirie M (2005) Genetics of anti-parasite resistance in invertebrates. Dev Comp Immunol 29: 9-32.
12. Granath WO, Yashino TP (1983) Lysosomal enzymes activities in susceptible and refractory snail groups of Biomphalaria glabrata during the course of infection with Schistosoma mansoni. J Parasitol 69: 1016-1026.
13. Loker ES, Bayne CJ (1986) Immunity to trematode larvae in the snail Biomphalaria. In "immune mechanisms in vertebrate vectors". (Lackie, A.M. Ed.) Oxford Univ Press New York pp 199-220.
14. Mohamed SH, Saoud MF, Rivera ER, Bruce Ji (1993) Granulocytes and hynalocytes in Biomphalaria alexandrina resisting infection with the Puerto Rican snail group of Schistosoma mansoni. Abst SRP Conf. Cairo Egypt Feb 17: 165.
15. Paraense WL, Corrêa LR (1963) Variation in susceptibility of population of Australorbis glabrat us to a strain of Schistosoma mansoni. Rev. Inst Med. Trop Sao Paulo 5: 15-22.
16. Loker ES, Bayne CJ (1982) In vitro encounters between Schistosoma mansoni primary sporocysts and hemolymph components of susceptible and resistant snail groups of Biomphalaria glabrata. Amer J Trop Med Hyg 31: 999-1005.
17. de Souza CP, Cunha RC, Andrade ZA (1995) Development of Schistosoma mansoni in Biomphalaria tenagophila, Biomphalaria straminea and Biomphalaria glabrata. Rev Inst Med Trop Sao Paulo 37: 201-206.
18. Chemin E (1970) Behavioral responses of miracidia of Schistosoma mansoni and other trematodes to substances emitted by snails. J Parasitol 56: 287-296.
19. Théron A, Pages JR, Rognon A (1997) Schistosoma mansoni Distribution patterns of miracidia among Biomphalaria glabrata snail as related to host susceptibility and sporocyst regulatory processes. Exp Parasitol 85: 1-9.
20. Yousif F, Ibrahim A, El-Bardicy SN (1998) Compatibility of Biomphalaria alexandrina, Biomphalaria glabrata and a hybrid of both to seven strains of Schistosoma mansoni from Egypt. J Egypt Soc Parasitol 28: 863-881.
21. Romeis B (1989) Mikroskopische Technik. Auflage, Urban & Schwarzenberg, Munich-Wien-Baltimore 17: 235-236.
22. Sokal RS, Rohlf FJ (1995) Biometry: The Principles and Practice of Statistics in Biological Research, W.H. Freeman and Co., New York, 887 pp.
23. Frandsen F (1979) Studies on the relationship between Schistosoma and their intermediate hosts. III. The genus Biomphalaria and Schistosoma mansoni from Egypt, Kenya, Uganda, West Indies and Zaire between two different strains from Katanga and Kishasha. J Helminthol 53: 321-348.
24. Bakry FA (2009) Genetic variation between Biomphalaria alexandrina and Biomphalaria glabrata snails and their infection with Egyptian strain of Schistosoma mansoni. J App Sci Res 5: 1142-1148.
25. Gandon S, Capowiez Y, Dubois Y, Michalakis Y, Olivier I (1999) Local adaptation and gene-for gene coevolution in a metapopulation model. Proc R Soc Lond B 263.
26. Lively CM (1999) Migration, virulence, and the geographic mosaic of adaptation by parasites. Amer Nat 153: 34-47.
27. Pan CT (1965) Studies on the host-parasite relationship between Schistosoma mansoni and the snail Aulocotrobus glabrat us. Amer J Trop Med Hyg 14: 931-976.
28. Pan SC (1980) The fine structure of miracidium of Schistosoma mansoni. J Invertebr Pathol 36: 307-372.
29. Mohamed AH (1998) Characterization of the hemocytes of Biomphalaria glabrata, Say. Gastropoda: Planorbidae. in interaction with two snail groups of Schistosoma mansoni Sambon 1907 Trematoda. Ph. D. Thesis. Fac. Biol. Tübingen Univ. Germany.
30. Théron A, Courtiau C (2005) Are Biomphalaria snails resistant to Schistosoma mansoni? J Helminthol 79: 187-191.
31. Loker ES, Bayne CJ, Buckley PM, Kruse KT (1982) Ultrastructure of encapsulation of Schistosoma mansoni mother sporocysts by hemocytes of juveniles of the 10-R2 snail group of Biomphalaria glabrata. J Parasitol 68: 84-94.
32. Sullivan JT, Richards CS (1981) S. mansoni, NIH-SM-PR-PR snail group in susceptible and non-susceptible stocks of B. glabrata Comparative histology. J Parasitol 67: 702-708.
33. Newton WL (1952) The comparative tissue reaction of two snail groups of Australorbis glabrat us to infection with Schistosoma mansoni. J Parasitol 38: 362-366.
34. McLaren DJ, Terry RJ (1982) The protective role of acquired host antigens during schistosomiae maturation. Parasite Immunol 4: 129-148.
35. Phillips TL, Shoulberg N, Gherson J (1984) Role of cellular and humoral components in the encapsulation response of Biomphalaria glabrata to Schistosoma mansoni sporocysts in vitro. Prog Clin Biol Res:17-29.
36. Adema CM, Hanington PC, Lun C, Rosenberg GH, Aragon AD et al. (2010) Differential transcriptional responses of Biomphalaria glabrata (Gastropoda, Mollusca) to bacteria and metazoan parasites, Schistosoma mansoni and Echinostoma paraensei (Digenea, Phyladhelminthes). Mol Immunol 47: 849-860.
37. Bayne CJ, Buckely PM, Dewan PC (1980) Schistosoma mansoni cytotoxicity of hemocytes from susceptible snail hosts for sporocysts in plasma from resistant Biomphalaria glabrata. Exp Parasitol 50: 409-416.
38. Loker ES, Bayne CJ, Yui MA (1986) Echinostoma paraensei hemocytes of Biomphalaria glabrata as targets of Echinostoma mediate interference with host snail's resistance to Schistosoma mansoni. Exp Parasitol 62: 149-154.
39. Sullivan JT, Hu PC (1995) A method for immunosolation of sporocysts of Schistosoma mansoni in non-susceptible snails. J. Parasitol 81: 1029-1031.
40. Adema CM, Loker ES (1997) Specificity and immunobiology of larval digenean-snail association. In "Advances in Trematode Biology", (Fried, B and Graczyk, T.K. Eds), CRC Press, Boca Raton : 229-263.
41. Lemos QT, Andrade ZA (2001) Sequential histological changes in Biomphalaria glabrata during the course of Schistosoma mansoni infection. Mem Inst Oswaldo Cruz 96: 719-721.
42. Guaraldo AM, Magalhães LA, Range HA, Pareja G (1981) Evolução dos esporocistos de Schistosoma mansoni Sambon, 1907, em Biomphalaria glabrata Say, 1818, e Biomphalaria tenagophila D'Orbigny, 1835,. Rev. Saúde Pública 15: 436-448.
43. Hahn UK, Bender RC, Bayne CJ (2001) Killing of Schistosoma mansoni sporocysts by hemocytes from resistant Biomphalaria glabrata: role of reactive oxygen species. J Parasitol 87: 292-299.
44. Bayne CJ, Hahn UK, Bender RC (2001) Mechanisms of molluscan host resistance and of parasite strategies for survival. Parasitology 123: 159-167.
45. Martins-Souza RL, Pereira CAJ, Coelho PMZ, Negrão-Corrêa D (2003) Silica treatment increases the susceptibility of the Cabo Frio snail group of Biomphalaria tenagophila to Schistosoma mansoni infection but does not alter the natural resistance of the Taim snail group. Parasitol Res 91: 500-507.