Size Resistance to Infection with the Schistosome Parasite in the Vector Snail

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Introduction

Schistosomiasis is a parasitic disease that affects over 207 million people in 74 countries, killing 200,000 annually (Lewis and Tucker, 2014). In the New World, the parasite develops in a freshwater snail, *Biomphlaria glabrata,* (Figure 1A) which releases larvae that penetrate human skin (Figure 1B).

Whereas most natural populations of *B. glabrata* are susceptible to infection, adults of the BS-90 strain of *B. glabrata*, originally isolated from Salvador, Brazil, kill the larval parasite with hemocytes (blood cells). However, Newton (1953) showed that neonatal BS-90 snails (less than 2-mm in shell diameter) were susceptible to infection.

As discussed by Chernin & Antolics (1975) the physiological mechanism for this neonatal susceptibility/adult resistance is unknown. Remarkably, no further research has been carried out on this topic during the ensuing 44 years. Because neonatal snails may be important in transmission of the parasite to humans, we examined the effect of snail size on parasite development and the immune response of the snail.

Figure 1A. *Biomphalria glabrata*

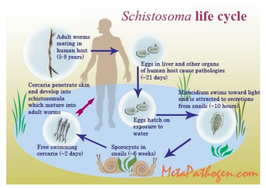


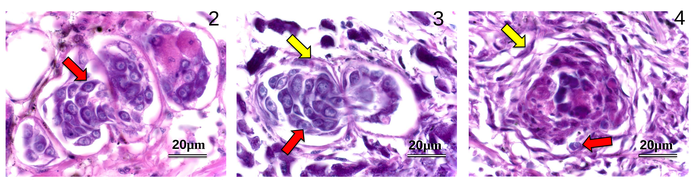
Figure 1B. Life Cycle of *Schistosoma* spp. http://www.geochembio.com

Materials and Methods

In order to test for the effect of size (shell diameter) on susceptibility, we used 463 BS-90 snails measuring 1, 2 , 3, 4, 5, 6, 7, and 8 mm. Each snail was exposed to 25 miracidia of *Schistosoma mansoni*. The snails were then incubated at 27˚ C for up to 30 days. We examined the snails weekly for infection using a dissecting microscope.

Subsequently, 50 BS-90 snails measuring 1, 2, 3, 4, and 5 mm were each exposed to 10 miracidia of *Schistosoma mansoni*. At 48 hr. post-exposure, we fixed the snails with Bouin's fluid, dehydrated the tissues in an isopropanol/xylene series, and embedded them in paraffin. Tissues were sectioned at 5-7 µm, and then stained with hematoxilin and eosin. We examined sections using a compound microscope at 1,000X, and counted mitotic figures in the snail's amoebocyte producing organ (APO) as a measure of its immune response. Additionally, we counted the number of germinal cells in each sporocyst (Figures 2-4). Finally, we determined whether the sporocysts were encapsulated by hemocytes and if so the number of layers of hemocytes surrounding each sporocyst (Figures 2-4).

We used several statistical tests. For the prevalence of infection data, we compared the proportion of infected snails of each size with that of 1-mm snails using the chi square test. We applied the Student's 2 tailed t-test in order to compare APO mitotic figures in parasite-exposed and unexposed snails of each size. For germinal cell counts, we used the chi square test to compare numbers in 2,3,4 and 5 mm snails to those in 1-mm snails. Values of $P < 0.05$ were considered statistically significant.



Figures 2-4. Sporocysts in snail tissue at 48 hr. post infection. 2, normal sporocyst. 3, encapsulated sporocyst. 4, destroyed sporocyst. Red arrows show germinal cells. Yellow arrows show encapsulated hemocytes.

Results

Prevalence of Infection

As shown in Figure 5, 1-mm snails were nearly fully susceptible. However, larger snails showed decreasing levels of susceptibility, and 4-mm snails were totally resistant to infection.

Germinal Cell Counts

Approximately 170 germinal cells per sporocyst occurred in 1-mm snails. As the snail grew in shell diameter, fewer germinal cells were found in each sporocyst (Figure 6). Germinal cells in all 5-mm snails were loose in the snail's tissue, having been released from disintegrating sporocysts.

APO Mitotic Figures

Mitotic activity in unexposed 1-mm snails was almost zero and slowly increased in larger snails (Figure 7). In miracidia exposed snails, mitotic activity did not increase in 1-mm snails but was significantly higher in the APO of larger snails relative to the exposed controls.

Encapsulation

Out of 47 sporocysts in the 1-mm snails, none were encapsulated by hemocytes. However, an increasing proportion of sporocysts were encapsulated in larger snails, with 100% of the sporocysts encapsulated in 5-mm snails. Not only was there a higher proportion of sporocysts encapsulated in the larger snails, but also the number of layers of hemocytes around each sporocyst increased (Figure 8).

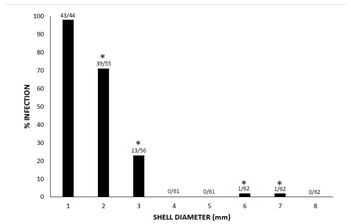


Figure 5. Percent infection with secondary sporocysts. Numbers above each bar shows the no. of snails infected/no. of snails exposed to the miracidia. *, $P < 0.05$ vs. 1-mm snail, chi square test.

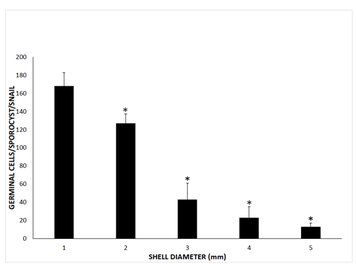


Figure 6. Mean (±SE) number of germinal cells per sporocyst at 48 hr. post-exposure *. $P < 0.05$ vs. 1-mm snail, chi square test. N = 10 snails in each size.

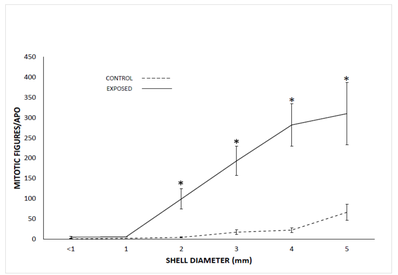


Figure 7. Mean (±SE) number of mitotic figures in amoebocyte producing organ in the unexposed snails (control) and miracidia-exposed snails at 48 hr. post infection. N = 10 *, significant difference between exposed and control snails, $P < 0.05$, 2-tailed t-test.

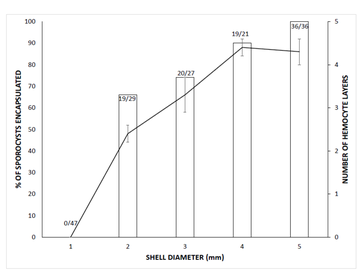


Figure 8. Bars show proportion of sporocysts surrounded by hemocytes at 48 hr. post exposure. Numbers above bars show no. encapsulated/total. Line shows mean (±SE) no. of layers of hemocytes surrounding encapsulated sporocysts.

Conclusions

- Susceptibility to infection rapidly declines as snails grow larger, with 1-mm snails exhibiting nearly complete susceptibility, while 4-mm snails show complete nonsuceptibility, thus confirming Newton's (1953) observation.
- As a snail grows in shell diameter, the immune system mounts a more effective response against the parasite.
  - An immune response occurs in the form of encapsulation by layers of hemocytes around a sporocyst. 1-mm snails show no signs of encapsulation. As the snail grows in shell diameter, there is an increasing proportion of encapsulated sporocysts and more layers of hemocytes around each sporocyst.
  - Mitotic activity in the APO of infected larger snails increases dramatically compared to uninfected snails. This result also suggests a stronger immune response in larger snails.
- The presence of germinal cells in a sporocyst is an indicator of its viability. Germinal cell counts are highest in 1-mm snails and decrease in larger snails. This result suggests that the snail's immune response negatively affects the parasite.

Literature Cited

1. Chernin E., & Antolics V.M. 1975. Neonatal susceptibility of Brazilian *Biomphlaria* to Puerto Rican *Schistosoma mansoni*. The Journal of Parasitology: 377-378.
2. Lewis F.A., & Tucker M.S. 2014. Schistosomiasis. Advances in Experimental Medicine and Biology: 47-75.
3. Newton W.L. 1953. The inheritance of susceptibility to infection with *Schistosoma mansoni* in *Australorbis glabratus*. The Journal of Parasitology: 247-248.

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