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COMPARATIVE STUDY OF THE MOTHER SPOROCYST OF *SCHISTOSOMA MANSONI* IN THE SUSCEPTIBLE AND RESISTANT SNAILS OF *BIOMPHALARIA GLABRATA*

I. B. KALHORO, S. JALALI[1] AND S. A. SHAMI

Department of Anatomy and Histology, Sindh Agriculture University, Tando Jam;
[1]Department of Biological Sciences, Quaid-I-Azam University, Islamabad, Pakistan

ABSTRACT

The comparative studies of the susceptible and resistant snails of *Biomphalaria glabrata* mass exposed to miracidia of *Schistosoma mansoni* were conducted from 1 to10 days post-exposure (DPE). Histological sections of 50 susceptible and 50 resistant snails revealed that many single, multiple, mature and migratory mother sporocysts were observed in the foot, head, lip, tentacle, mantle, anus, buccal mass, neck, kidney, oesophagus, respiratory epithelium of the lung and pericardial cavity of the heart of susceptible snails. Whereas, few single and multiple mother sporocysts were visible in the earlier mentioned first eight organs of the resistant snails throughout infection period. Single mother sporocyst was located in the foot, head, lip and tentacles of susceptible snails at 1-2 DPE. At 3 DPE, multiple mother sporocysts were found in the above organs, and very few of them were observed in the mantle and muscles of the anus of these snails. In the resistant snails, such mother sporocysts were only found in the tentacle and columellar muscles at 9 DPE. At few mother sporocysts reached the buccal mass of the susceptible snails at 4 DPE. Increasing in the number of the single and multiple mother sporocysts were observed in the foot, head and tentacles, whereas a few of them were also visible in the neck and kidney of these snails at 5 DPE. Most of the mother sporocysts grew further in the foot, head, tentacles, mantle and kidney and developed into the mature form at 6 DPE onwards. At 8 DPE, some mature mother sporocysts were observed in the above mentioned organs and oesophagus of these snails. In the foot, head, lip and tentacle at 8 DPE, tegument of mature mother sporocyst was ruptured due to the increase number, and size of the embryos, and a few of them migrated towards the deeper tissues of the organs of the body of the snails. At 9 and 10 DPE, most of the above types of mother sporocyst remained in the earlier mentioned organs, very few were also found in the pericardial cavity of the heart and respiratory epithelium of the lung of the susceptible snails. Chi-square test results showed highly significant differences in the total infected snails with mother sporocyst in the various organs of the susceptible and resistant snails.

Key words: *Schistosoma mansoni***,** mother sporocyst, Biomphalaria, snails, susceptible, resistant.

INTRODUCTION

Biomphalaria glabrata is the most prevalent vector of the diseases due to the high levels of compatibility and susceptibility to the parasites (Souza *et al*., 1995). Schistosomes require a molluscan intermediate host in which they not only undergo a series of developmental stages but also asexual reproduction (Cheng and Bier, 1972). *Schistosoma mansoni* miracidia are attracted by water in which *B. glabrata* snails have been kept and penetrate in to the body surface of snail by a lytic enzyme secreted from the apical glands or the gut (Wajdi, 1966). Miracidia are infective forms for the snail host, differentiating into sporocysts immediately after penetration. During the course of its life cycle, *S. mansoni* goes through distinct stages of differentiation (Wajdi, 1966). Mother sporocysts were found in the head-foot and tentacles of the snails (Wajdi, 1966; Lewis *et al*., 1993). Three snails species viz. *B. glabrata*, *B. tenagophilia* and *B. straminea*, which had been infected with *S. mansoni* miracidia revealed similar qualitative features, consisting of areas of sporocyst proliferation and associated with reactive host reaction (Souza *et al*., 1997). The mother sporocysts can survive longest in the foot, head and tentacles of the snails (Pan, 1965). The present study was designed for the comparison of distribution, location and migration of *S. mansoni* mother sporocysts in the susceptible and resistant snails of *B. glabrata*.

MATERIALS AND METHODS

Selected pigmented resistant and susceptible ordinary (Albino) snails of *B. glabrata*, the intermediate host of *S. mansoni*, were studied for normal parasite development. The shell diameter of snails of both

groups was measured which was between 10.22 ± 1.02 and 9.0 ± 0.91 mm, respectively. They were mass infected with freshly hatched miracidia of *S. mansoni* in the laboratory of School of Biological Sciences, University of North Wales, Bangor United Kingdom. The experimental animals were twice mass exposed with miracidia obtained from the livers of mice. A total of 50 susceptible and 50 resistant snails were histologically processed from 1 to 10 days post-exposure (Kalhoro, 1998).

The infected snails were relaxed in 0.4% Nembutal anaesthesia (Abbot Laboratories) for 8-24 hours. Relaxed snails were fixed in Heidenhain's Susa fixative until shell became transparent. The shell of the snails was carefully removed (without any damage to the tissues of the snails) with artery forceps under dissecting microscope.

For histological studies, snail bodies were dissected into anterior and posterior regions, which included areas as the head-foot, tentacles, mantle cavity and digestive gland and ovotestis. The snails were dehydrated in graded alcohol, infiltrated and embedded in historesin. Four micrometer thick serial sections were cut with a glass knife from animals using 5 repeats on every day and fixed on glass slides. The slides were stained using polychrome method (Blackstock, 1998). The stained slides were mounted with Dextrene Plasticizer and Xylene (DPX) and a cover slip and dried on a hot plate over night. Finally, the stained slides were examined under a light microscope. Microscopic examination of the tissues of the snails and larval stages of the parasite was performed on serial sections (Kalhoro, 1998).

Total number of infected snails and mother sporocysts observed in the various organs were counted. Chi-square test was used to see the magnitude of difference in number of mother sporocysts between resistant and infected snails.

RESULTS

The involvement of the organs, number of the infected snails and total mother sporocysts found in the various organs of the susceptible and resistant snails are shown in Table 1. After penetration by the miracidia, mother sporocysts were found in 12 different organs of the susceptible and 9 organs of the resistant snails. The highest number (50) of the infected animals and mother sporocysts were recorded in the foot (239), followed by head (125), and tentacles (105) of the susceptible snails. Whereas, in the resistant group, maximum number (35) of the infected snails and mother sporocysts were present in the foot (11) and tentacles (15).

Moreover, at 1 and 2 DPE, mother sporocysts were visible in the foot, head, lip and tentacle, while in mantle and anus of the susceptible snails at day 3. After 4 and 5 DPE, these migrated through the muscles of the buccal mass, neck and reached the kidney of these snails. Mother sporocysts further developed in most of the above organs and oesophagus; at 9 and 10 DPE, a few of them were also visible in the pericardial cavity of the heart and respiratory epithelium of the lung of the susceptible snails. Similarly, in the resistant snails, mother sporocysts remained in the anterior organs (foot, head, lip, tentacle, mantle, anus, buccal mass and neck) with a few of them were recognized in the columellar

Table 1: Involvement of organs, number of infected snails and total *S. mansoni* mother sporocysts found in various organs of the susceptible and resistant snails of *B. glabrata*

DPE	Organ	Infected snails		No. of mother sporocysts	
		Susceptible	Resistant	Susceptible	Resistant
1-10	Foot	50	35	239	11
	Head	50	25	125	6
	Lip	35	5	61	1
	Tentacle	50	35	105	15
3-10	Mantle	30	15	77	4
	Anus	20	5	13	1
4-10	Buccal mass	25	10	18	2
5-10	Neck	25	10	43	2
	Kidney	15	-	10	-
8	Oesophagus	1	-	1	-
9	Heart	1	-	1	-
	Columellar muscles	-	2	-	7
10	Lung	1	-	3	-

DPE = Days post-exposure; - = Not visible

Table 2: Chi-square test applied on the mother sporocysts present in the similar organs of the susceptible and resistant snails 1-10 DPE.

Snails	Foot	Head	Lip	Tentacle	Mantle	Anus	Buccal mass	Neck	Total
Susceptible	239	125	61	105	77	13	18	43	681
	(235.48)	(123.39)	(58.40)	(113.03)	(76.29)	(13.19)	(18.84)	(42.39)	
Resistant	11	6	1	15	4	1	2	2	42
	(14.52)	(7.61)	(3.60)	(6.97)	(4.71)	(0.81)	(1.16)	(2.61)	
Total	250	131	62	120	81	14	20	45	723

($\Sigma X^2_{(7)}$ 21.76, P< 0.001)

muscles of the resistant snails at 9 DPE. Most of the susceptible snails showed increasing number of mother sporocysts in majority of the organs, while lesser number of the parasites was seen in the resistant group. The total number of the mother sporocysts found in the similar organs of the snails were statistically analysed (Table 2). The chi-square test showed highly significant difference between the mother sporocysts found in the susceptible and resistant snails ($\Sigma X^2_{(7)}$ 21.76, P< 0.001).

Location of the mother sporocyst in the various organs of the snails

The exact anatomical locations of the mother sporocysts found in various regions of the organs of susceptible and resistant snails on 1-10 days post-exposure (DPE) are presented in Table 3. Mother sporocysts were found in the anterior surface of the foot of the susceptible snails at 1, 2, 7 and 10 DPE. A few of them were found in the posterior surface of the foot of these snails on 9 DPE. At 3-6 and 8 DPE, many of the mother sporocysts were visible on the both surfaces of the foot of susceptible snails. Mother sporocysts remained in the foot of the snails from 1 to 10 DPE. During these days single, multiple and mature types of the mother sporocysts were found in these snails. A few mother sporocysts were found on the anterior surface of the foot of resistant snails at 1, 3 and 6 DPE and on the posterior surface on 5 and 10 DPE. On both surfaces of the foot of resistant snails, they were observed on 4 and 9 DPE. No mother sporocysts were seen on the foot of these snails 2, 7 and 8 DPE.

Table 3: Location of *S. mansoni* mother sporocysts in some organs of the susceptible and resistant snails of *B. glabrata*

DPE	Snails	Foot		Head		Lip		Tentacle		
		Ant	Post	Ant	Post	Ant	Post	Apex	Body	Base
1	S	+	-	+	+	+	-	-	+	+
	R	+	-	4	-	-	-	-	-	+
2	S	+	-	-	+	-	-	-	+	+
	R	-	-	-	-	-	-	-	+	-
3	S	+	+	+	-	+	-	+	+	-
	R	+	-	+	-	-	-	-	+	-
4	S	+	+	+	-	-	+	-	+	+
	R	+	+	-	-	-	-	-	+	-
5	S	+	+	+	+	+	+	-	+	+
	R	-	+	+	-	-	-	-	+	-
6	S	+	+	+	+	+	+	-	+	+
	R	+	-	-	--	-	-	-	-	-
7	S	+	-	+	-	-	-	-	-	+
	R	-	-	-	-	-	-	-	+	-
8	S	+	+	+	+	+	-	+	+	+
	R	-	-	-	-	-	-	-	-	-
9	S	-	+	+	-	+	+	-	+	-
	R	+	+	+	-	-	-	-	+	-
10	S	+	-	+	+	-	-	-	+	+
	R	-	+	+	-	+	-	-	-	-

- = Not visible, DPE = Days post-exposure, Ant = Anterior, Post = Posterior, R = Resistant, S = Susceptible.

In the head of the susceptible snails, mother sporocysts were found on the anterior surface of the head of the susceptible snails at 3, 4, 7 and 9 DPE. A few of them were found on the posterior surface of the head of these snails on 2 DPE. At 1, 5, 6, 8 and 10 DPE, many of the mother sporocysts were visible on both surfaces of the head of susceptible snails. Mother sporocysts remained in the head of the snails from 1 to 10 DPE. During these days, single, multiple and mature types of the mother sporocysts were found in these snails. Whereas, a few mother sporocysts were found only on the anterior surface of the head of the resistant snails at 1, 3, 5, 9 and 10 DPE.

In the lip of the susceptible snails, mother sporocysts were found on the anterior surface of the lip of the susceptible snails at 1, 3 and 8 DPE. A few of them were found on the posterior surface of the lip of these snails on 4 DPE. At 5, 6 and 9 DPE, some mother sporocysts were visible on both surfaces of the lip of susceptible snails. During these days single, multiple and mature types of the mother sporocysts were found in these snails. No mother sporocysts were visible in the lip of the snail on 2, 7 and 10 DPE. Whereas, single mother sporocyst was found only on the anterior surface of the lip of the resistant snails at 10 DPE.

In the tentacles of the susceptible snails, many mother sporocysts remained in the apex, body and base at 8 DPE. A few of them were seen in the former two regions (apex and body) of the organ at 3 DPE. On 1, 2, 4-6 and 10 DPE, mother sporocysts were visible in the latter two regions (body and base) of the tentacles of the snails. In the body, mother sporocysts were observed at 3 and 9 DPE and in the base of the tentacle a few mother sporocysts were visible on day 7 only. Mother sporocysts remained in the tentacles of the snails from 1 to 10 DPE. During these days single, multiple and mature types of the mother sporocysts were found in these snails. In the tentacles of the resistant snails, mother sporocysts were found in the body on 2-5, 7 and 9 DPE and in base only at 1 day post-exposure.

In the mantle of the susceptible snails, mother sporocysts were found on the anterior surface at 3, 5 and 7 DPE. A few of them were found in the posterior surface of the mantle of these snails on 8 and 10 DPE. At 6 DPE, some mother sporocysts were visible on the both surfaces of the mantle of susceptible snails. During these days, single, multiple and mature types of the mother sporocysts were found in these snails. No mother sporocyst was visible in the mantle of the susceptible snail on 1, 2, 4 and 9 DPE. Whereas, mother sporocyst was found only on the anterior surface of the mantle of the resistant snails at 10 DPE. On the posterior surface of the mantle of these snails, mother sporocyst was found on 6 and 9 DPE. Additionally, some mother sporocysts were located on the muscles of the anus, buccal mass, neck and oesophagus of the susceptible snails from 3 to 8 DPE. In the resistant snails, a few mother sporocysts were found in the columellar muscles at 9 DPE. Very few mother sporocysts were found in the respiratory epithelium of the lung of the susceptible snails at 10 DPE. In these snails, a few mother sporocysts were found in the connective tissues of the kidney and heart at 5 and 9 DPE.

DISCUSSION

This study describes distribution, location and migration of the mother sporocysts of *S. mansoni* found in the susceptible and resistant snails. Several different host-parasite interactions occur with different combinations of *B. glabrata* stocks and *S. mansoni* strains (Sullivan and Richards, 1981). These interactions are influenced by genes of the snails that control susceptibility and genes of the parasite that determine infectivity. Some gene combinations allow the parasite to develop and proliferate because the snail fails to recognise it as a foreign body (Lewis *et al*., 1993). Only four or five mother sporocysts developed normally in the susceptible snails exposed to 25 miracidia after 24 hours (Richards, 1975). In the present study, 10 mother sporocysts developed in the foot, head, lip and tentacles of the susceptible snails mass exposed to miracidia of *S. mansoni* after 1 DPE.

Mother sporocysts have been found in the foot-head areas of snails (Sullivan and Hu, 1996; Ataev *et al*., 1997). In the present study, after penetration of *S. mansoni* miracidia, mother sporocysts were found in the above organs, also in the lip and tentacles of the susceptible snails at 1 DPE. Pan (1965) also reported that the mother sporocysts survived longest in the foot, head and tentacles of the snails. However, mother sporocysts were also visible in the lip, mantle and anus of the susceptible snails. Mother sporocysts have been reported in the tentacles of the snails by Wajdi (1966), Lewis *et al*. (1993) and Ataev *et al*. (1997).

In the present study, mother sporocysts were observed on the muscular tissues of the buccal mass of the susceptible and resistant *B. glabrata* snails after 4 to 10 DPE. Ataev *et al*. (1997) also reported that mother sporocysts of *E. caproni* developed in the muscular tissues of the buccal mass of *B. pfeifferi* snails. Pan (1965) observed a few mother sporocysts in the posterior portion of the kidney of the *A. glabratus* snails at 1, 2, 4, 8 and 11 DPE. In the present study, mother sporocysts were seen in the connective tissues of the kidney of the susceptible snails at 5, 6 and 10 DPE. Ataev *et al*. (1997) found that mother sporocysts of *E.*

caproni developed in the mantle epithelium of the *B. pfeifferi* snails. These findings are in close agreement with the present study where from 3 DPE onwards mother sporocysts were present in the anterior and posterior surfaces of the mantle of the susceptible snails. In resistant snails these were also observed in this organ at 6, 9 and 10 DPE. Pan (1965) reported a few mother sporocysts in the columellar muscles of the *A. glabratus* snails. But in the present study, 2 to 9 mother sporocysts were also found in the columellar muscles of the resistant snails at 9 DPE. Pan (1965) reported the number of mother sporocysts found in the head-foot (82), tentacles (20) and kidney (6) of the *A. glabratus* snails at 8 DPE.

Conclusions

On the basis of the above findings it can be concluded that infection developed more in the susceptible and less in the resistant snails. Increase number and variable shapes of the mother sporocysts of *Schistosoma mansoni* developed earlier in certain organs of the susceptible snails. However, it was not seen in the resistant snails of *Biomphalaria glabrata*.

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