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"Comparative Studies on the Susceptible and Non-Susceptible *Biomphalaria alexandrina* the Intermediate Snail Host of *Schistosoma mansoni* in Western Saudi Arabia"

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Abstract: Schistosomiasis is an important parasitic disease that infects humans. Among the main species of schistosomes infecting humans is *Schistosoma mansoni* and *S. haematobium*. Snails of various genera, such as *Biomphalaria alexandrina* and *Bulinus truncates*, act as intermediate hosts and play a major role in the transmission of schistosomes. Transmission from human to snail is under the influence of the ciliated miracidium larvae that hatch from the eggs of the parasite voided in the faeces or urine. After a period of asexual multiplication in the snail, a second aquatic larval stage, the cercaria, emerges and infects human by direct penetration of the skin. The present work was carried out on *Biomphalaria alexandrina* snails, the intermediate host of *Schistosoma mansoni*. Snails were collected from various sites in south western Saudi Arabia. Their progeny were reared in the laboratory under standard conditions. Different biological experiments were carried out to determine the susceptibility level in the various populations of snail. The miracidia were obtained by isolating viable ova of the hatching parasite from faeces of infected individuals (human and mice). Different sets of regulated experimental infection of snails with the miracidia of *Schistosoma mansoni* were preformed. The effect of miracidial density, water temperature, miracidial age, size of snails, as well as light and darkness were investigated. The study revealed that the infection rate increased as the number of miracidia used increased (1, 60%; 2, 60%; 3, 70%; 5, 80%; 15, 90%; and 20, 90%). No significant increase of infection rate was achieved at higher water temperatures (at 15°C: 65% with newly hatched miracidia; 75%, with 15 minutes miracidia; 85% with 30 minutes miracidia; 95% with 45 minutes miracidia and 100% with 60 minutes miracidia. At 20°C: 75% with newly hatched miracidia; 80%, with 15 minutes miracidia; 90%, with 30 minutes miracidia; 95% with 45 minutes miracidia and 100% with 60 minutes miracidia). Similar results were obtained at 25°C and 30°C. However, the infection rate decreased in relation to snail size increase. At snail size 2-4 mm the infection rate was 90%, the rate decreased to 85% at 4-6 mm and 6-8 mm size, while at 8-10 mm size the rate was 80%. At 10-12 mm and 13-15 mm size the rate dropped to 75%. Light and dark conditions had a significant difference on the infection rate (40% in darkness and 90% in light). Laboratory observations also indicated that some of the snails exposed to infection with miracidia of the respective parasite developed infection and produced cercariae, while the rest remained uninfected.

Key words: *Biomphalaria alexandrina* · intermediate host · susceptible snails · non-susceptable snails · Schistosomiasis · *Schistosoma mansoni* · miracidia · Saudi Arabia

INTRODUCTION

Schistosomiasis is a disease caused by infection with various species of the genus *Schistosoma.* Some 200 million people are probably infected and 500-600 million more are exposed to infection [1]. The infection is transmitted by specific aquatic snails in a wide variety of fresh water habitat. Among the species of schistosomes infecting humans are *Schistosoma mansoni* and *S. haematobium. Biomphalaria alexandrina* and *Bulinus truncatus* are the intermediate hosts for *S. mansoni* (agent for intestinal schistosomiasis) and *S. haematobium* (agent for urinary schistosomiasis).

Urinary and intestinal schistosomiasis is endemic in Saudi Arabia. In 2004, the prevalence of schistosomiasis was 2.9/100,000 persons (0.0029%) according to the

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Saudi Arabia Ministry of Health Statistic Book, (http://www.moh.gov.sa/statistics/1425/Default.html). According to the source, a total of 1192572 persons were examined, 639 were infected (0.05%). The prevalence among Saudis was 61.2% and non Saudis 38.8%. Males have higher infection rate (82%) than females (18%). The rate of infection was higher among the 15-39 year-old age group (53.7%). The highest prevalence was reported in Jazan, Bishah, Aseer and Al-Bahah. Urinary schistosomiasis is prevalent in Jazan, Aseer and Bishah, whereas intestinal schistosomiasis is prevalent in Taif, Al-Bahah, Aseer, Bishah, Najran, Makkah Al-Mukarramah, Al-Medina and Hail. A total of 34305 water sources were examined in different localities in the above mentioned areas in the year 2004 and 778 (~2.3%) were found to be contaminated. Limited studies have been conducted to explore the susceptibility and none- susceptibility among snails in Saudi Arabia. Lwambo *et al*. [2] studied the infectivity of miracidia of the Saudi Arabian isolate of *S. mansoni* in *Biomphalaria arabica* and found that the snails are influenced by several factors such as miracidial dose, water temperature and salinity. Arfaa *et al*., [3] assessed the potential role of three species of *Bulinus* in the transmission of *Schistosoma haematobium* in Saudi Arabia, on the basis of their susceptibility to experimental infection, their geographical distribution and numbers and type of habitats in which they were found.

Previous studies showed that some snails that were exposed to infection with miracidia of schistosomes developed infection and produced cercariae, while the others remained uninfected. Many documented data indicated that many external and internal factors influence the number of trematode larvae produced by their intermediate snail hosts with special reference to schistosomes [4]. Among the variables studied are the temperature [2,5,6], host nutritional status [7], genetic differences within the parasite population [8], the life span of the snail [9] and the size of the snails [4,6]. Studies also showed that the susceptibility of snails to schistosome's infection depends on the metabolic status of the snail itself. One of the metabolic activities depends upon the production of reactive oxygen species by hemocytes from the snail [10,11]. Professional phagocytes play a crucial ruole in host defense against pathogens. Their arsenal includes the ability to initiate a respiratory burst [12,13]. The generation of reactive oxygen species is apparently essential for efficient killing of bacteria and fungi [14,15]. DeGaffé and Loker [16] correlated the susceptibility of the snail *Biomphalaria glabrata*to to infection with the digenetic trematode *Echinostoma paraensei* with the ability of secretory–excretory products (SEP) derived from sporocysts of this parasite to interfere with the spreading behavior of host hemocytes in an *in vitro* assay. Some studies did not detect strain differences [17-20] Guillou *et al.* [21] stated the presence of susceptibility or resistance of *B. glabrata* to the trematode *Echinostoma caproni.* Mascara *et al.* [22] carried out artificial selection of *Biomphalaria tenagophila* snails for susceptibility to infection by *Schistosoma mansoni* (Brazilian SJ strain) from natural populations. Occasionally, non-susceptible snails outbreed the susceptible ones and replace them [23,24].

The detection of *Biomphalaria* snails infected with *S. mansoni* was usually performed by cercariae shedding induced by artificial light exposure or by squeezing snails between two glass slides. However, these methods are not able to detect the parasite neither in dead snails nor in the pre-patent period. Accordingly, infection diagnosis is only possible after the parasite has completed its life cycle (3 to 4 weeks after infection), when cercariae release is started [4].

The aim of this study was to investigate the susceptibility of *Biomphalaria* snails exposed to experimental infection with *S. mansoni* influenced by different factors including: miracidial density, water temperature, miracidial age, size of snails, as well as miracidial invasion in light and dark conditions. The existence of susceptible and non-susceptible strains of snails will be assessed. Such investigations are important implication for field control studies. Hence, it will be valuable to breed non-susceptible snails in large scale and introduce them into the field. Natural selection would further act to increase the proportion alleles for insusceptibility and eventually provide some mean of biological control for schistosomiasis in natural populations.

MATERIALS AND METHODS

Snails and parasites:

(a) Rearing of snail intermediate hosts: 100 snails were collected from different localities and habitats (irrigation canals and drains, current stream, ponds, dry canals etc...) in Southwestern region of Saudi Arabia and examined individually for cercarial production. Snails were reared singly in either a 250 ml or 400 ml beaker with a Petri dish cover at an ambient temperature at 26°C. Tap water aerated for at least 24 hours was used and snails were fed dried oven or fresh lettuce 2 or 3 times a week. Reproduction was by self-fertilization. Rooms, in which

snails were maintained and experiments were conducted, were kept at a temperature of about 26°C. Eggs were laid by adult snails of the first generation. Each snail progeny to be isolated from rearing was numbered from "1" on maturity is based on onset of egg lying. Each snail's progeny were maintained under standard laboratory conditions as described above to give the second and third generation of snails which were subjected to the same biological studies.

(b) Production of miracidia: Snails were exposed to miracidia hatched from eggs in the faeces of mice and/or human infected with trematodes. Fresh faeces were comminuted in aerated tap water, allowed to settle for 5 minutes then fresh water was added. The procedure being repeated until the supernatant was clear. The sediment was then examined under the dissecting microscope and as miracidia hatched they were transferred individually by pipette to the snail containers.

Experimental Methods:

1- Miracidial density and the rate of infections: Single snails of 10-15 mm shell diameter were exposed in 5 ml of water at 26°C for a period of 30 min to a range of miracidial densities (1, 2, 3, 5, 15 and 20 miracidial / 5ml). The miracidia used in these experiments have an average age of 30 minutes, twenty snails were used for each of these miracidial densities. After exposure, snails were removed, placed in rectangular plastic container (45 x 28 x 13 cm) and kept for 27 - 50 days later to assess whether or not they had acquired infection through the observation of cercaria shedding.

2- Temperature, miracidial age and miracidial infectivity: Freshly hatched miracidia were pipetted into dishes and left to age under a range of temperatures. The miracidial age dependent infectivity was carried out at the following temperatures: 15°C, 20°C, 25°C and 30°C. At each regulated temperature, the miracidia infectivity were estimated using 5 different age groups of miracidia (freshly hatched, 15, 30, 45 and 60 min). Five miracidia of a given identical age were randomly chosen and placed in 5 ml of regulated water container. One snail of 10-15 mm in diameter was then added to the experimental vessel and exposed to infections for 2 hours. A total of 20 snails were used for each miracidial age. After exposure, the snails were transferred to rectangular plastic containers at 26°C constant temperature. From the 27th to the 50th day, snails were examined for infections through the observation of cercaria shedding. Snails that fail to shed cercariae by the 50th day after exposure were crushed and examined microscopically for infection.

3- Size dependent host susceptibility to infection: Snails of varying shell sizes (2-4, 4-6, 6-8, 8-10, 10-12 and 13-15 mm) were exposed to 10 miracidia, average age of 30 min, in 5 ml of water at 26°C for a period of 1 hour under conditions of light. Twenty snails from each size group were exposed. Snails were examined from the 27th day up to the 50th days to assess whether or not they had acquired infection through the observation of cercaria shedding.

4- Illumination and darkness and the rate of infection: Twenty snails of 10-12 mm (shell diameter) were exposed individually to 5 miracidia. The infection was carried out in the presence of electric lamp (100 watt) for 10 hours. Similarly, another twenty snails of 10-12 mm were exposed in complete darkness. After miracidial exposure, each group was transferred to a labeled plastic container and maintained under same laboratory conditions. Snails were monitored for a total period of 50 days for cercarial shedding starting by the 27th day after infection.

RESULTS

Out of the 100 field collected snails only 15 snails were naturally infected with *Schistosoma mansoni* (15%).

1- Miracidial density and the rate of infections: The infection rate of snails exposed to different miracidial density is summarized in Table 1. Although the results showed that the rate of infection increases as the number of invading miracidia increase yet it was statistically insignificant. However, the value of P = 0.9 (not significant).

2- Temperature and miracidial age effect on infectivity: The results obtained from exposing snails to different aged miracidia at different temperature are summarized in Table 2. Generally, temperature and miracidial age had an insignificant effect on snail infectivity rate. However, snails exposed to the freshly hatched, 15 and 30 minutes aged miracidia displayed higher infection at 15°C and 20°C. Higher temperatures (25°C and 30°C) generated the same infection rate. Snails exposed to 45 and 60 minutes aged miracidia showed the same infection rate at the different temperature tested.

3- Size dependent host susceptibility to infection: The results obtained from exposing different sized snails to the same number of miracidia (10) are shown in Table 3.

Table 1: Miracidial density in relation to the rate of infection of snails

Total number (No.) of snails used	120 snails (20 snails each)					
Miracidial densities	1	2	3	5	15	20
No. of susceptible snails	12	12	14	16	18	18
No. of non-susceptible snails	8	8	6	4	2	2
Rate of infection (%)	60 %	60 %	70 %	80 %	90 %	90 %

Table 2: Number of infected snails with different ages of miracidia at different temperature

No. of total snails used	100 snails (20 snails each) Age of miracidia				
Water temperature	Freshly hatched	15 min.	30 min.	45 min	60 min.
Number of snails infected at 15°C	13 (65 %)	15 (75 %)	17 (85 %)	19 (95 %)	20 (100 %)
Number of snails infected at 20°C	15 (75 %)	16 (80 %)	18 (90 %)	19 (95 %)	20 (100 %)
Number of snails infected at 25°C	15 (75 %)	16 (80 %)	18 (90 %)	19 (95 %)	20 (100 %)
Number of snails infected at 30°C	15 (75 %)	16 (80 %)	18 (90 %)	19 (95 %)	20 (100 %)

Table 3: The infection rate in relation to snail size

Number of exposed snails	120 snails (20 snails each)					
Size of snails	2-4mm	4-6mm	6-8mm	8-10mm	10-12mm	13-15mm
No. of infected snails	18	17	17	16	15	15
Rate of infection (%)	90 %	85 %	85 %	80 %	75 %	75 %

Table 4: Rate of infection of snails exposed to miracidia at light and dark periods

Number of total snails	40 snails (20 snails each)	
Illumination	Light	Darkness
Number of infected snails	18	8
Rate of Infection (%)	90 %	40 %

Results indicated that the smaller the snail size the higher the infection rate. This was also non-significant statistically.

4- Illumination and darkness and the rate of infection: The results obtained from exposing snails to the same number of miracidia (5) under light and dark periods are shown in Table (4). Results showed that the infection rates were significantly higher in light condition than in the dark.

DISCUSSION

Schistomiasis occurs in Saudi Arabia (Saudi Arabia Ministry of Health Statistic Book, 2004, http://www.moh.gov.sa/statistics/ 1425/ Default.html). The present work was intended to investigate the susceptibility and non-susceptibility of the *Biomphalaria* snails, the intermediate host of *Schistosoma mansoni*, to experimental infection in relation to different external variables which may affect its rate of infection with the parasite. The infection rate among the snails collected from the field was (15%). Snail susceptibility to infection was confirmed only when each snail was tested individually for production of cercariae, usually from the 27th to the 50th day (~3-7 weeks) after infection thus allowing enough time for the parasite to completed its life cycle.

The suggestion of susceptible and non-susceptible snails to infection was expressed by a number of authors [10-18,20-23,25-27]. The relation between snail's infectivity and the density of miracidia is well documented. Previous studies reported higher infection rate as miracidial dose increased [28,29,6]. The results obtained from this study also showed that the snail's infection rate increased as the number of miracidia was increased, in spite of being not statistically significant. Loker [30] exposed laboratory-reared *Lymnaea catascopium* snails individually to different numbers of *Schistosomatium douthitti* miracidia. Increasing the exposure dosage from 3 to 10 miracidia generally increased infection rates, in some age classes up to 100%.

Snail's susceptibility to infection affected by miracidial age under different degrees of temperatures regulated water. Infection occurred at all miracidial ages influenced by different water temperatures. Regardless, of being statistically insignificant; the lowest infection rate was encountered among the freshly hatched miracidium exposed at 15°C; while the rate of infection was among the (60 minutes) aged miracidium at the different temperature examined (15, 20, 25 and 30°C). Anderson *et al.* [5] reported that the death rate of miracidia declined exponentially with age where life-expectancy was maximal (approximately 16 h) at 15°C. Infectivity also declined rapidly with larval age but, in contrast to larval survival, the rate of infection was at its maximum at 25°C. Lwambo *et al.* [2] found that water temperature during exposure had an influence on the mortality, infection rate and cercarial production in *Biomphalaria arabica* exposed to *S. mansoni* miracidia.

The infection rate was highest in snails exposed at 28 and 34°C. No infection of *Biomphalaria arabica* occurred at the temperature of 10°C. Shoukry *et al*. [6] indicated that snails 4-6 mm in diameter exposed to 5 freshly hatched miracidia under light and in water at 25°C were the optimum conditions for infection of *Biomphalaria alexandrina*.

Age and size-dependent susceptibility was presented by earlier workers [4,6,29-32]. The present work suggested that snail's susceptibility to be non-significantly correlated with its size. This data confirm and extend earlier work on snail's susceptibility and indicate that the susceptibility declined with increased snail size. The infection rate was at its highest among snails 2-4 mm in diameter exposed to 20 snails. This may be related to the ability of larger snails to kill invading miracidia, or the possibility of a nutritional competition between parasite and snail. Such competition for nutrients has been studied mostly in association with a reduction in egg laying during invasion of the hepatopancreas by secondary sporocysts [33]. Anderson *et al.* [5] indicated that snail susceptibility was shown to be more closely correlated with host size rather than host age. The susceptibility declined exponentially with increased host size. Size-dependent susceptibility was shown to generate concave age-prevalence curves for infection within snail populations, where the maximum prevalence is generated in snails of intermediary age. Niemann and Lewis [4] used *Biomphalaria glabrata* snails of the same age, but different sizes, to determine size-related susceptibility to *Schistosoma mansoni* miracidial infection and the influence of snail size on total cercarial production. Snails with shell diameters from <5 to >17 mm were individually exposed to miracidia. The percentage of snails which developed infections was lower in snails with larger shell sizes.

The current work also showed that snail's susceptibility was higher under light conditions which was also previously reported by Shoukry *et al*. [6].

In conclusion, this work demonstrated differences in *Biomphalaria* snail's susceptibility in relation to various biological factors. Insusceptibility to infection with *Schistosoma mansoni* could be a heritable character. Joubert *et al.* [34] proposed the use of insusceptible snails as a possible method of controlling schistosomiasis. He also suggested that to change the susceptibility of natural snail populations from being predominantly susceptible to a non-susceptible state was, through the release of refractory snails into natural habitats. Finally, it would be beneficial to select non-susceptible snails and mass culture them to increase the proportion of the alleles of insusceptibility as a possible means for biological control of schistosomiasis in natural population. Further investigation is needed to elucidate the reason of susceptibility and insusceptibility in snails.

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