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Notification of Acceptance of the Abstract entitled:

Comparative Studies on the Susceptible and Non-Susceptible *Biomphalaria alexandrina* the intermediate snail hosts of *Schistsomiasis mansoni* in Western Saudi Arabia"

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Dear Dr Manal
We are pleased to inform you that your abstract has been accepted and selected for oral presentation in the 3rd Saudi Science Conference organized by King Saud University to be held during March 10-13th , 2007 at King Fahad Cultural Centre, Riyadh. Oral presentation time would be 15 minutes including 5 minutes for discussion for each paper. Presenting author (or at least one of the author) should be registered in the conference.
Looking forward to welcoming you in Riyadh for your participation in the conference.
With best compliments and regards

Sincerely

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LITERATURE REVIEW

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 (Webbe, 1981). 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 Saudi Arabia Ministry of Health Statistic Book, 2004, (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 year 2004, 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.* (1987) studied the infectivity of miracidia of the Saudi Arabian isolate of *S. mansoni* in *Biomphalaria arabica* and found that to be influenced by several factors as miracidial dose, water temperature and salinity. Arfaa *et al.*, (1988) 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 develop infection and produce 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 (Niemann and Lewis, 1990). Among the variables studied are the temperature (Anderson *et al.*, 1982, Lwambo *et al.*, 1987; Shoukry *et al.*, 1997), host nutritional status (Coles 1973), genetic differences within the parasite population (Cohen and Eveland 1988), the life span of the snail (Frandsen 1979) and the size of the snails (Niemann and Lewis, 1990; Shoukry *et al.*, 1997). 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 (Bender *et al.*, 2005; Sasaki *et al.*, 2005). Professional phagocytes play a crucial rule in host defense against pathogens. Their arsenal includes the ability to initiate a respiratory burst (Conners and Yoshino, 1990; Hampton *et al.*, 1998). The generation of reactive oxygen species is apparently essential for efficient killing of bacteria and fungi (Reeves *et al.*, 2003; Roos and Winterbourn, 2002). DeGaffé and Loker (2002) correlated the susceptibility of the snail *Biomphalaria glabrata*to infection with the digenetic trematode *Echinostoma paraensei* was 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 (Dikkeboom *et al.*, 1988a, 1988b, Bayne *et al.*, 1988 and Hahn *et al.*, 2000. Guillou *et al.* (2004) stated susceptibility or resistance of *B. glabrata* to the trematode *Echinostoma caproni.* Mascara *et al.* (2002) 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 (Shozawa *et al.*, 1989; Mkoji *et al.*, 1990).

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 (Niemann and Lewis, 1990).

The aim of this study is 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 laid by adult snails of the first generation. Each snail progeny to be isolated form 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 about 5 minutes and fresh water 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. The containers used in these experiments are circular flat-bottomed Petri dish of 16 mm in diameter. After exposure, snails were removed placed in rectangular plastic container (45 x 28 x 13cm) and kept for 27 - 50 days later to assess whether or not they have 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 temperature. The miracidial age dependent infectivity was carried out at the following temperatures: 15°C, 20°C, 25°C and 30°C. Water temperature was regulated by the use of a thermostatically controlled water bath. 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 (described above) 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 27^{th} to the 50^{th} 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 have 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.

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

Number (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.
No. of snails infected at 15 °C	13 (65 %)	15 (75 %)	17 (85 %)	19 (95 %)	20 (100 %)
No. of snails infected at 20 °C	15 (75 %)	16 (80 %)	18 (90 %)	19 (95 %)	20 (100 %)
No. of snails infected at 25°C	15 (75 %)	16 (80 %)	18 (90 %)	19 (95 %)	20 (100 %)
No. of snails infected at 30 °C	15 (75 %)	16 (80 %)	18 (90 %)	19 (95 %)	20 (100 %)

3- Size dependent host susceptibility to infection:

The results obtained from exposing different sized snails to the same number of miracidia (10) were shown in table (3). Results indicated that the smaller the snail size the higher the infection rate. However, the value of P = 0.9 (not significant).

Table 3: The infection rate in relation to snail size

Number of exposed snails	120 snails (20 snails each)					
Size of snails	2-4 mm	4-6 mm	6-8 mm	8-10 mm	10-12mm	13-15 m
Number of infected snails	18	17	17	16	15	15
Rate of infection (%)	90 %	85 %	85 %	80 %	75 %	75 %

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). The results showed that the rate of infection increases as the number of invading miracidia increase. However, the value of P = 0.9 (not significant).

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 %**

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 have insignificant effect on snail infectivity rate, the value of P = 0.68. However, snails exposed to the freshly hatched, 15 and 30 minutes aged miracidia displayed higher infection at 15 degree C and 20 degree C temperatures; while higher temperature (25 ˚C & 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.

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 is 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 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 (Dönges, 1974; Kassim and Richards, 1979; Paraense, 1988; Dikkeboom *et al.*, 1988a; Dikkeboom *et al.*, 1988b; Shozawa *et al.*, 1989; Conners and Yoshino, 1990; Hampton *et al.*, 1998; Hahn *et al.*, 2000; DeGaffé and Loker, 2002; Mascara *et al.*, 2002; Reeves *et al.*, 2002; Roos and Winterbourn, 2002; Guillou *et al.*, 2004; Bender *et al.*, 2005 and Sasaki *et al.*, 2005). The relation between snail's infectivity and the density of miracidia is well documented. Previous studies reported higher infection rate as miracidial dose increased (Carter *et al.*, 1982; el-Assal *et al.*, 1997; Shoukry *et al.*, 1997). 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 (1978) 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%.

4- Illumination and darkness, and the rate of infection:

The results obtained from exposing snails to same number of miracidia (5) under light and dark periods were shown in table (4). Results showed that the infection rates were higher in light condition than in the dark. The value of P = 0.1, is slightly significant

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 %**

Snail's susceptibility to infection affected by miracidial age in different temperature 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 degrees C; while the rate of infection was among the (60 minutes) aged miracidium at the different temperature examined (15, 20, 25, 30 degrees C). Anderson *et al.* (1982) reported that the death rate of miracidia declined exponentially with age where life-expectancy was maximal (approximately 16 h) at 15 degrees C. Infectivity also declined rapidly with larval age but, in contrast to larval survival, the rate of infection was at a maximum at 25 degrees C. Lwambo *et al.* (1987) 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 degrees and 34 degrees C. No infection of *Biomphalaria arabica* occurred at the temperature of 10 degrees C. Shoukry *et al.*, (1997) 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 (Loker, 1978; Niemann and Lewis, 1990; Cooper *et al.* (1994); el-Assal *et al.*, 1997; Shoukry *et al.*, 1997; Zakikhani and Rau, 1999). The present work suggested that snail's susceptibility to be correlated with its size, though it's not statistically significant. 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 the 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 (Crews and Yoshino 1989). Anderson *et al.* (1982)

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 (1990) 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, similar finding was reported by Shoukry *et al.*, (1997).

In conclusion, this work that demonstrated differences in *Biomphalaria* snail's susceptibility in relation to various biological factors. Variations in snail susceptibility were encountered. Insusceptibility to infection with *Schistosoma mansoni* could be a heritable character. Joubert *et al.* (1990) 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, 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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