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Susceptibility of *Biomphalaria* spp. to infection with *Schistosoma mansoni* in sympatric and allopatric combinations with observations on the genetic variability between snails

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ABSTRACT

This investigation was carried out to study the susceptibility of Saudi *Biomphalaria arabica* to Egyptian *Schistosoma mansoni* in comparison with the susceptibility of Egyptian *Biomphalaria alexandrina* to the same parasite. This was in order to know the possibility that the parasite might be able to spread into Saudi Arabia and to determine the genetic variability between Egyptian *B. alexandrina* and Saudi *Biomphalaria arabica* snails. Lab bred Egyptian *B. alexandrina* and Saudi *B. arabica* snails were exposed individually to 10 freshly hatched Egyptian *S. mansoni* miracidia/snail. The mortality rate, infection rate, prepatent period, duration of cercarial shedding and cercariae production per snail were recorded in both the sympatric couple (Egyptian *B. alexandrina* and Egyptian *S. mansoni*) and in the allopatric combination (Saudi *B. arabica* and Egyptian *S. mansoni*). The results revealed that, the survival rate of snails exposed to Egyptian *S. mansoni* miracidia at 34th day post-exposure (at first cercarial shedding) was higher in *B. arabica* than in *B. alexandrina*. After shedding, the mortality rate was higher in the *B. arabica*, compared to *B. alexandrina*. The infection rate was higher in *B. arabica* than *B. alexandrina*; the mean of prepatent period was shorter in the *B. arabica* than in the *B. alexandrina*. However, the duration of cercarial shedding was longer in the Egyptian snails and the cercarial production per snail was higher in *B. alexandrina* snails than in *B. arabica*. To study the genetic variability between *B. alexandrina* and *B. arabica*, RAPD-PCR on the genomic DNA of snails was done. RAPD-PCR revealed significant variation between the two snail species. In conclusion, the results suggest that *B. arabica* can play a role in the transmission of Egyptian *S. mansoni* in Saudi Arabia and therefore this parasite might be able to spread into the Kingdom. In addition, the RAPD-PCR results demonstrated genetic variability between the two species which may be related to the differences in susceptibility of both Saudi and Egyptian *Biomphalaria* snails to Egyptian *S. mansoni* infection.

1. Introduction

Schistosomiasis is a water-based disease which is considered the second most important parasitic infection after malaria in terms of public health and economic impact. Schistosomiasis is endemic in 76 countries. Of the 207 million people with schistosomiasis, 85% live in Africa. Other regions affected are the Americas (Brazil, Suriname and

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Venezuela, as well as several Caribbean islands); the Eastern Mediterranean (Islamic Republic of Iran, Iraq, Saudi Arabia, Syrian Arab Republic and Yemen); and eastern Asia (Cambodia, China, Indonesia, Japan, Lao People's Democratic Republic and the Philippines) (WHO Fact Sheet, 2010).

Egypt and Saudi Arabia, two neighboring Arabian countries have many mutual economic interests and a large number of Egyptian workers-specially farmers- temporary migrate to Saudi Arabia for working in agriculture activities (Statistical Year Book of the Ministry of Work in Saudi Arabia, 2008). Since, Egypt is one of the most severely affected countries with schistosomiasis (El-Khoby et al., 2000) and the intermediate hosts of schistosomes: *Biomphalaria arabica*, *Bulinus truncates*, *Bulinus beccarii* and *Bulinus wrighti* snails are available in Saudi freshwater bodies (Arfaa, 1976; Arfaa et al., 1989), therefore study of the susceptibility of such Saudi snails to Egyptian schistosomes appeared very interested to determine the possibility of spreading of these parasites in Saudi Arabia which already suffered from schistosomiasis with prevalence rate 2.78/100,000 individuals (Statistical Year Book of the Ministry of Health in Saudi Arabia, 2008).

Numerous studies have demonstrated geographic variation in susceptibility of snails to schistosome infection since snails appeared less susceptible to geographically distant strains of the parasite or completely refectory to these strains (Wajdi et al., 1979; Arfaa et al., 1989; Manning et al., 1995; Mukaratirwa et al., 1996; Southgate et al., 2000; Njiokou et al., 2004; Mostafa et al., 2009; Borda and Rea, 2010). Many authors suggested that variability in susceptibility may be caused by genetic factors (Anderson and May, 1979; Mulvey and Vrijenhoek, 1982; Oliveira et al., 2008).

Several attempts have been made to determine the genetic variability among schistosomiasis intermediate hosts by using protein electrophoresis, allozyme phenotypes analysis and other molecular biology techniques (Mulvey and Woodruff, 1985; El-Khayat et al., 2008). Recently, several investigators showed that random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) is useful for distinguishing the genetic differences between and within different *Biomphalaria* snail species (Abdel-Hamid et al., 1999; Knight et al., 1999; Oliveira et al., 2008).

The present work aimed to study the susceptibility of Saudi *B. arabica* to Egyptian *Schistosoma mansoni* in comparison with the susceptibility of Egyptian *Biomphalaria alexandrina* to the same parasite in order to know the possibility of spreading of this parasite in Saudi Arabia, as well as to determine the genetic variability between Saudi and Egyptian *Biomphalaria* snails on the molecular level.

2. Materials and methods

2.1. Snails

Lab bred Egyptian *B. alexandrina* were obtained from the Medical Malacology laboratory at Theodor Bilharz Research Institute (TBRI) in Egypt. Saudi *B. arabica* snails were collected from freshwater bodies in Abha. The city of Abha, the capital of Asser Province in southwestern Saudi Arabia, lies in the high mountains of Asser, at an altitude of about 2250 m above sea level, and approximately 200 km from the northern border of Yemen. The mean annual temperature at Abha (latitude 17°80 N, longitude 42°46 E) reached 18.5 °C, with maximum (22 °C) during June, July and August; and minimum (12 °C) during December and January. The rainfall is expected at any time of the year with maximum (150 mm) during May and minimum (2 mm) during December. Saudi snails were transferred to Egypt and maintained in the Malacology laboratory at TBRI for breeding and production of lab bred snails. The first generation of such snails was used in the present investigation to avoid any stress which might result from transportation of snails from KSA to Egypt and to be sure that the snails were clean and free from any pathogens.

2.2. Schistosome egg production

Ten golden hamsters were injected intraperitoneally with 100 ± 10 Egyptian *S. mansoni* cercariae per hamster. Hamsters and cercariae were purchased from the Schistosome Supply biological Program (SBSP) at TBRI in Egypt. Six weeks post-exposure, hamsters were sacrificed and dissected to obtain the liver and intestine. Hepatic and intestinal tissues were cut into pieces, placed in 0.85% saline solution and homogenized. The suspension was poured into a column of sieves arranged in descending order of mesh opening (420 μm, 177 μm, 105 μm and 45 μm). The eggs were collected from the bottom sieve, suspended in dechlorinated tap water and exposed to light to stimulate hatching of miracidia.

2.3. Susceptibility test

Lab bred Egyptian and Saudi snails (4–5 mm in shell diameter) were exposed individually to 10 freshly hatched *S. mansoni* miracidia/snail. Exposure to miracidia was carried out for about 3 h in 0.5 ml dechlorinated tap water per snail at 26 °C ± 1 °C. Following, snails were washed thoroughly and maintained in aquaria under laboratory conditions. From 25 days post-exposure, exposed snails were placed individually in small plastic vials (each containing about 10 ml of water and a small piece of lettuce leaf) and examined day after day for cercarial shedding by exposing them to artificial light for 2 h at 25 °C. After the 1st observed cercarial shedding, snails were tested weekly to determine the cercarial productivity. The emission of cercariae was followed until death in all positive specimens. The experiment was ended 89 days post-exposure. The mortality rate, infection rate, incubation period, duration of cercarial shedding and cercarial production per snail were recorded in both the sympatric couple (Egyptian *B. alexandrina* and Egyptian *S. mansoni*) and in the allopatric combination (Saudi *B. arabica* and Egyptian *S. mansoni*).

2.4. Statistical analysis

The obtained parasitological data were subjected to Student's *t*-test using SPSS version 8 to determine statistical significance.