Veterinary Parasitology 161 (2009) 207–212

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Susceptibility of Saudi *Bulinus truncatus* to infection with Egyptian *Schistosoma haematobium* with observations on protein electrophoretic pattern of the snails

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ARTICLE INFO

Article history:
Received 11 May 2008
Received in revised form 28 November 2008
Accepted 4 December 2008

Keywords:
Bulinus truncatus
Schistosoma haematobium
Schistosomiosis
Susceptibility
Egyptian
Saudi
Abha
Highland
Protein electrophoresis
Sympatric
Allopatric

ABSTRACT

A laboratory-based susceptibility study was carried out on snails *Bulinus truncatus* collected from highland Abha, Asser, Saudi Arabia to Egyptian *Schistosoma haematobium* to investigate the potential role of Saudi *B. truncatus* in the transmission of Egyptian *S. haematobium* and to know the possibility that the parasite might be able to spread into Saudi Arabia. The results revealed that, compared to Egyptian snails, survival of snails at day 25 post-exposure was significantly higher in Saudi *B. truncatus* ones. The infection rate was higher in Saudi snails as compared to Egyptian ones. The incubation period was shorter in Saudi snails but the duration of cercarial shedding was longer in the Egyptian than in the Saudi snails. The production of *S. haematobium* cercariae per snail was higher in Egyptian snails than in Saudi ones. These results suggest that Saudi *B. truncatus* can play a role in the transmission of Egyptian *S. haematobium* in Saudi Arabia and therefore this parasite might be able to spread into the Kingdom. In addition, electrophoretic analysis of tissue soluble proteins was done to determine the effects of the parasite on both the Egyptian and Saudi snails. The electrophoretic analysis revealed the occasional presence or absence of certain bands in infected snails in comparison with non-infected one.

1. Introduction

Schistosomiosis or bilharziosis is one of the most prevalent helminth infections in the world as it is endemic in about 75 countries that cause a serious public health concern in the developing world. The transmission cycle requires specific freshwater snails as intermediate hosts (Engels et al., 2002).

According to the Health Statistical Year Book of the Ministry of Health in Saudi Arabia (2006) the prevalence rate of bilharziosis in the Kingdom of Saudi Arabia (KSA) was 2.2/100,000; the percentage of urinary bilharziosis was 33.4% while that of the intestinal bilharziosis was 66.6% and no case of combined bilharziosis was recorded. 62.9% of cases of bilharziosis have been detected among Saudi individuals in comparison to 37.1% among non-Saudi persons. The highest rates of bilharziosis in Saudi Arabia were recorded in Al-Bahah (26.2%), Asser (21.9%), Jazan (21.2%) and Bishah (12.8%). The lowest rate was recorded in Tabouk (0.4%). No case has been recorded in Riyadh and Al-Jouf.

It has been found that the snail *Biomphalaria arabica* acts as the intermediate host for *S. mansoni* in Saudi Arabia (Arfaa, 1976). For *S. haematobium*, on the other hand, three species of snails namely, *Bulinus truncates*, *B. beccarii* and *B. wrighti*, have been incriminated as intermediate hosts (Arfaa et al., 1989).

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doi:10.1016/j.vetpar.2008.12.026

In Saudi Arabia, 271,839 non-Saudi individuals work in agriculture and fisheries activities; a large number of them are Egyptian farmers (Statistical Year Book of the Ministry of Work in Saudi Arabia, 2005). Egypt is one of the most severely schistosomiosis affected countries. El-Khoby et al. (2000) collected health questionnaires and parasitological examinations of urine and stools from a stratified random sample of 89,180 individuals from 17,172 households in 251 rural communities in 9 Egyptian governorates; they reported that the prevalence of *S. haematobium* ranged from 0.3 to 13.7 and that of *S. mansoni* ranged from 42.9 to less than 1.

Several investigations were made to study the compatibility between *Schistosoma* spp. from different localities and different populations of intermediate host snails. The susceptibility of Saudi *B. truncatus* to the infection with Egyptian *S. haematobium* was studied by Arfaa et al. (1989); they demonstrated that the infection rate was 3% and the snails were "susceptible" only when exposed to pooled miracidia and appeared "non-susceptible" when exposed to 10 miracidia. Wajdi et al. (1979) studied the susceptibility of Iraqi *B. truncatus*, *Gyranaulus ehrenbergi*, *Physa fontinalis*, *Lymnaea lagetis*, *Melanoides tuberculata* and *Melanopsis nodes* to the infection with Egyptian *S. haematobium* and *S. mansoni*; they reported that Egyptian *S. haematobium* but not Egyptian *S. mansoni* infect Iraqi *B. truncatus* and both proved to be unable to infect any of the other snails included in their study. In order to estimate the risk of extension of urinary schistosomiosis in Cameroon, Njiokou et al. (2004) studied the compatibility between *S. haematobium* from three remote localities (Mourtourwa, Gounougou and Kékem) and four populations of *B. truncatus* (Gounougou, Ngaoundéré, Bertoua and Kékem) and four populations of *B. globosus* (Mourtourwa, Ouroudoukoudje, Bafia and Yaoundé); they suggested that *B. truncatus* might be potentially more implicated than *B. globosus* to the extension of the urinary bilharziosis in Cameroon.

The development of the intramolluscan larval stages of the parasite within the parasitized snails altered the electrophoretic profile of plasma and soluble tissue proteins of the snails. Gress and Cheng (1973) followed up the changes in total serum proteins and protein fractions in *B. glabrata* parasitized by *S. mansoni* from 1 h to 70 days post-exposure at 14 time intervals. Loker and Hertel (1987) reported on the alteration of *B. glabrata* plasma due to *Echinostoma paraensei* infection. Zelck et al. (1995) analyzed the plasma proteins of *B. glabrata* in the presence and absence of *S. mansoni*. El-Ansary et al. (2000) studied the tissue protein profiles of *B. alexandrina* infected with *S. mansoni*. Moreover, the effect of double infection with *S. mansoni* and *E. liei* on some physiological parameters such as glucose, glycogen, protein and enzyme concentrations in *B. alexandrina* was studied by Sharaf El-Din and El-Sayed (2001) and El-Dafrawy et al. (2001). Mostafa et al. (2001) studied the influence of *S. mansoni* and/or *E. liei* infection on the tissue soluble proteins of *B. alexandrina*. The influence of *Fasciola gigantica* and *S. haematobium* infection on the tissue soluble proteins of *L. natalensis* and *B. truncatus* was studied by Mostafa (2002). Electrophoretic patterns of protein fractionations in haemolymph and tissues of *B. alexandrina* and *B. truncatus* during course of schistosome infection were followed by El-Dafrawy et al. (2006).

The present study aimed to investigate the potential role of Saudi *B. truncatus* in the transmission of Egyptian *S. haematobium* and to study the effect of *S. haematobium* on the tissue soluble proteins of *B. truncatus* from both Egypt and Saudi Arabia, using SDS-PAGE technique.

2. Materials and methods

2.1. Snails

Lab bred Egyptian snails *B. truncatus* were obtained from Schistosome Supply Biological Program (SBSP) at Theodor Bilharz Research Institute (TBRI) in Egypt. Saudi *B. truncatus* snails were collected from freshwater bodies in Abha, Asser district, Saudi Arabia. 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. It has the lowest mean annual temperature compared with any of the southern urban areas, a low atmospheric pressure of oxygen and a high annual rainfall with rain falling mainly in winter and spring. Saudi snails were maintained in the Malacology laboratory at TBRI in Egypt for breeding and production of lab bred snails; the first generation of such snails was used in the present investigation to avoid the stress resulted 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 eggs production

Ten mice of CD1 strain were exposed to 100 ± 10 cercariae of Egyptian *S. haematobium* per mouse by the tail immersion method, modified by Oliver and Stirewalt (1952). Mice and cercariae were purchased from SBSP unit at TBRI in Egypt. Six weeks post-exposure, mice 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, 177, 105 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 of Egyptian and Saudi *B. truncatus* snails (4–5 mm in shell height) were exposed individually to 10 freshly hatched *S. haematobium* miracidia/snail. Exposure to miracidia was carried out for about 3 h in 0.5 ml dechlorinated tap water per snail under 26 ± 1 °C, and then snails of each group were washed thoroughly and maintained in a separate aquarium under laboratory conditions. One group of Egyptian snails and other group of Saudi snails were kept uninfected as control. From 25th day 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 daily for cercarial shedding by