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Susceptibility of Five Populations of Jordanian *Bulinus truncatus* to Infection with Nigerian, Ghanian and Egyptian Strains of *Schistosoma haematobium*

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Abstract

The susceptibility of five *Bulinus truncatus* snail populations originating from Jordan to infection with three strains of *Schistosoma haematobium* from Nigeria, Ghana and Egypt was determined. The results showed that all five populations are susceptible to these parasite strains and have the ability to become infected with a wide variety of *S. haematobium* strains from different geographic areas of the Middle East and Africa.

Key words: *Bulinus truncatus*, Jordan, Susceptibility, *Schistosoma haematobium*.

Introduction

The discovery of *Bulinus truncatus*, the snail intermediate host of *Schistosoma haematobium* was relatively recent in Jordan. The snail species was reported for the first time from a site in the Jordan Valley in 1975 (Saliba et al., 1976) and then from an ancient Roman pool near the town of Jarash in 1978 (Saliba and Salameh, 1980). Additional breeding sites for the snail intermediate host were reported after an extensive survey of other freshwater bodies (Bruce, 1984).

The status of schistosomiasis in Jordan until recently was quite different from other endemic countries for the disease, in that despite the presence of *B. truncatus* vector snails, there were no records for indigenous schistosomiasis haematobia cases. An extensive mass urine examination of human populations living in close proximity to the areas infested by the snail intermediate hosts revealed no positive cases (Saliba et al., 1980, Bruce and Burch, 1983).

Recently, Saliba et al. (1986) reported 27 indigenous cases during 1984-1985 from Karak Governorate, southern Jordan. In addition, they located water bodies which were newly infested with the vector snail.

Previous reports indicated that Jordanian *B. truncatus* populations were found to be susceptible to Egyptian strains of *S. haematobium*. Saliba et al. (1981) exposed *B. truncatus* from Muthalth Al-Masri and Jarash in Jordan, with Egyptian strain of *S. haematobium* which had been maintained in the laboratory by passage through hamsters for 23 years. Ayed and Saliba (1985) exposed five populations of *B. truncatus* from Jordan, with the *S. haematobium* isolated from Egyptian workers harboring the parasite. They found that all Jordanian *B. truncatus* snail populations were susceptible to the parasite.

Various investigators have pointed out that different populations of the intermediate snail host from different geographical regions exhibited variations in their susceptibility when they were exposed to different geographical strains of the schistosome parasite (Files and Cram, 1942; McCullough, 1959; Webbe and James, 1971; Lo, 1972).

Since the compatibility of different populations of *Bulinus* snails to geographic strains of

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S. haematobium is known to vary, our knowledge of the susceptibility of the Jordanian strain of *B. truncatus* should not be limited only to its compatibility for the Egyptian strain of *S. haematobium,* but should rather be extended to include other African and Middle Eastern strains of *S. haematobium.* The present study was therefore carried out to determine the susceptibility of 5 populations of *B. truncatus* from Jordan to Nigerian, Ghanian and Egyptian (new isolate) strains of *S. haematobium.*

Materials and Methods

I. Source of Snails and Parasite Strains.

The Jordanian snail populations used in this study are progeny of parent stock maintained in the laboratory of Dr. E. Saliba (Department of Biological Sciences, University of Jordan), originally collected from various sites in Jordan during the period of 1980-1983 (Fig. 1). Some of these progeny snails were brought to the Center for Tropical Diseases, University of Lowell, as breeders in August 1983 and have been maintained continuously since that time.

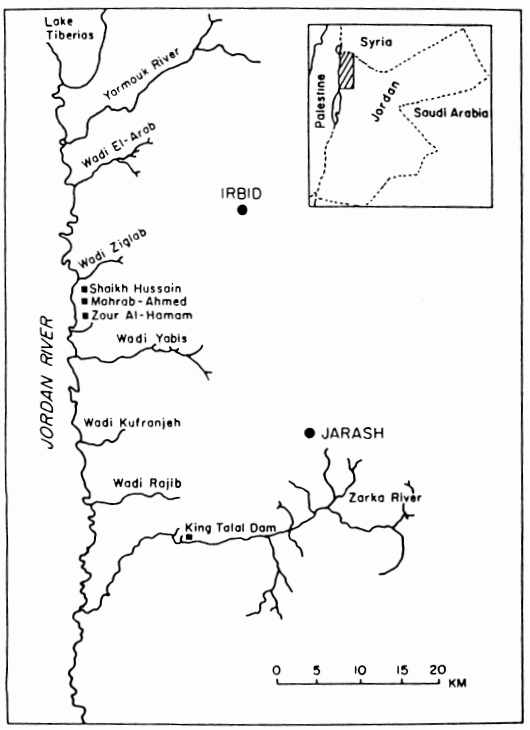

Fig. 1 Map of Jordan, indicating the study area.

Three strains of *S. haematobium* were used:

1. *Ghanian strain:* This strain was isolated by Dr. H. van der Schalie in 1974 then transferred to the Center for Tropical Diseases, University of Lowell in 1977, where it has been maintained continuously in the laboratory since that time. The snail intermediate host used to maintain this strain is *Bulinus truncatus rohlfsi* which was originally collected from Anyabani near Lake Volta in 1974.
2. *Nigerian strain:* This strain was isolated by Dr. H. van der Schalie in 1974 and was transferred to the Center for Tropical Diseases, University of Lowell in 1977. The snail intermediate host used to maintain this strain is *B. t. rohlfsi,* from Nigeria.
3. *Egyptian strain:* This strain of schistosome is a relatively new isolate obtained by Dr. Yung-san Liang, Center for Tropical Diseases, University of Lowell from Abu Rawash, near Cairo, Egypt in 1981. The snail intermediate host used to maintain this strain was also obtained from the same locality in 1980.

II. Snail Cultivation and Maintenance.

Snails were cultivated and maintained according to Liang (1974a, 1974b).

III. Collection of Miracidia.

Schistosome haematobium eggs were obtained from the large intestines of hamsters *(Cricetus auratus)* with mature *S. haematobium* infection exposed to cercaria 120 days previously. After hamsters were sacrificed by injecting them with 0.1 ml of sodium pentobarbital (1 gr./ml), their large intestines were removed and rendered free of both fat and fecal material and then cut into small pieces. The small pieces of intestine were placed in an Eberbach stainless steel container with approximately 20 ml of physiological saline (0.85%). The stainless steel container was then placed on a Waring blender which was connected to a variable autotransformer and the intestinal tissue was homogenized for 15 seconds at very

low speed (30 volts). The homogenate was then passed through a series of sieves (425 μ, 177 μ, 106 μ and 45 μ) to collect eggs. The eggs were washed through to the bottom sieve (45 μ) with 100 ml of 0.85% saline. This procedure was repeated three times at low (50 volts), medium (70 volts) and high speed (100 volts) respectively. Aerated tap water was then used to render the eggs free from saline. The eggs were washed from the bottom sieve into a Petri dish and concentrated in the center of the dish by gentle rotation. The eggs were then collected by using a pasteur capillary pipette, and placed into a small Petri dish (1.5 X 6.0 cm). Within a few minutes, the eggs usually began to hatch, yielding active miracidia (Liang and Kitikoon, 1980). The miracidia were collected by using a finely drawn pasteur capillary pipette.

IV. Exposure of Snails.

Snails 2-3 weeks of age were exposed either individually or en masse for three hours. The exposure dose was either 5 miracidia/snail or 10 miracidia/snail. After exposure, snails were transferred into Petri dishes containing mud and algae, and maintained for the duration of the experiment in 12/12 hours dark/light period.

V. Determination of Infection.

Five weeks after exposure, snails were examined individually to check for infection with *S. haematobium*. Prior to examination, the Petri dishes containing exposed snails were covered with a dark cloth. On the following morning, snails were dried for 10 to 15 minutes, and then placed in shedding vials (2.0 X 3.0 cm) containing 2-3 ml of filtered tap water under bright illumination for three hours. Infected snails were identified by visualization of cercarial emergence in the water by use of a dissecting microscope. All snails found to be negative were examined separately for three successive weeks and then crushed to determine the presence of sporocysts.

VI. Analysis of Data.

A chi-square test of association was used to determine if there were differences in infection rates among the five populations, separately for each strain of the parasite.

Results

The susceptibility of 5 *Bulinus truncatus* populations from Jordan following exposure to three African strains of *Schistosoma*

Table 1 Survivorship and infection rates of 5 populations of Jordanian *Bulinus truncatus* snails to the Egyptian strain of *Schistosoma haematobium* (5 miracidia/snail/individual)

Snail population	No. exposed	Exposed snails: Survival No.	Survival %	Infected No.	Infected %	Unexposed snails: Survival No.*	Survival No.†	Survival %
Zarka River	66	60	91.0	21	35.0	66	62	93.9
Zour Al-Hamam	66	53	80.0	4	7.5	66	61	92.9
Shaikh Hussain	66	63	95.4	18	28.6	66	63	95.4
Mahrab Ahmed	33	26	78.9	7	26.9	66	60	90.9
King Talal Dam	33	30	90.9	12	40.0	66	62	93.9
Infection control *B. truncatus* from Egypt	66	66	100	23	34.8	66	64	97.0

* No. of snails at week 0.
† No. of snails at week 5.

haematobium is presented in Tables 1, 2, 3 and 4.

All five populations of Jordanian snails were found to be susceptible to the Egyptian strain of the parasite (Table 1 and 2). The infection rates ranged from 7.5% (Zour Al-Hamam) to 40.0% (King Talal) when snails were exposed individually to 5 miracidia/snail. Survivalship rates for exposed snails ranged from 78.9% (Mahrab Ahmed) to 95.4% (Shaikh Hussain) (Table 1). The infection rates for snails exposed to 10 miracidia/snail/en masse ranged from 52.4% (Mahrab Ahmed) to 20% (Zour Al-Hamam), and the survival rates for the exposed snails ranged from 75.8% (Mahrab Ahmed) to 98.4% (Shaikh Hussain). Unexposed snail colony control survival rate was 93.9% (Table 2).

For the Ghanian strain, all five populations were susceptible to infection (Table 3). The

Table 2 Survivorship and infection rates of 5 populations of Jordanian *Bulinus truncatus* snails to the Egyptian strain of *Schistosoma haematobium* (10 miracidia/snail/in masse)

Snail population	No. exposed	Exposed snails: Survival No.	Survival %	Infected No.	Infected %	Unexposed snails: Survival No.*	Survival No.†	Survival %
Zarka River	66	52	78.8	24	46.2	66	62	93.9
Zour Al-Hamam	66	55	83.3	11	20.0	66	61	92.9
Shaikh Hussain	66	65	98.4	26	40.0	66	63	95.4
Mahrab Ahmed	33	25	75.8	13	52.4	66	60	90.9
King Talal Dam	33	31	93.9	15	48.4	66	62	93.9
Infection control *B. truncatus* from Egypt	66	65	98.5	33	50.8	66	64	97.0

* No. of snails at week 0.
† No. of snails at week 5.

Table 3 Survivorship and infection rates of 5 population of Jordanian *Bulinus truncatus* snail to the Ghanian strain of *Schistosoma haematobium* (5 miracidia/snail/en masse)

Snail population	No. exposed	Exposed snails: Survival No.	Survival %	Infected No.	Infected %	Unexposed snails: Survival No.*	Survival No.†	Survival %
Zarka River	63	53	84.1	10	18.9	66	61	92.4
Zour Al-Hamam	60	32	80.0	9	28.1	33	31	93.9
Shaikh Hussain	45	38	84.4	8	21.1	33	30	90.9
Maharb Ahmed	63	48	76.2	11	22.9	66	60	90.9
King Talal Dam	73	59	80.1	13	22.0	66	62	93.9
Infection control *B. t. rohlfsi* from Ghana	83	65	70.9	17	26.2	66	63	95.4

* No. of snails at week 0.
† No. of snails at week 5.

Table 4 Survivorship and infection rates of 5 populations of Jordanian *Bulinus truncatus* snails to the Nigerian strain of *Schistosoma haematobium* (5 miracidia/snail/en masse)

Snail population	No. exposed	Exposed snails: Survival No.	Survival %	Infected No.	Infected %	Unexposed snails: Survival No.*	Survival No.†	Survival %
Zarka River	70	55	78.6	19	34.5	66	60	90.9
Zour Al-Hamam	85	66	77.6	23	34.8	66	61	92.4
Shaikh Hussain	81	66	81.5	17	25.6	66	62	93.9
Mahrab Ahmed	130	92	70.8	21	22.8	66	61	92.4
King Talal Dam	66	56	84.5	13	23.2	66	63	95.4
Infection control *B. t. rohlfsi* from Nigeria	83	70	84.3	19	27.1	66	62	94.2

* No. of snails at week 0.
† No. of snails at week 5.

infection rate ranged from 18.9% (Zarka River population) to 28.1% (Zour Al-Hamam population). Survivalship rates for exposed snails ranged from 76.2% (Mahrab Ahmed snails) to 84.4% (Shaikh Hussain snails). Unexposed survival rates ranged from 90.9 – 93.9%.

The five Jordanian *B. truncatus* populations were susceptible to the Nigerian strain of the parasite (Table 4). The infection rate ranged from 22.8% (Mahrab Ahmed snails) to 34.8% (Zour Al- Hamam snails) when exposed to 5 miracidia/snail/individually. Survival of exposed snails were 70.8% (Mahrab Ahmed snail) to 84.5% (King Talal Dam snails). Survival of unexposed snails were 90.9% (Zarka River snails) and 95.4% (King Talal Dam snails).

Discussion

The results obtained from exposure of snails of each of five populations of *Bulinus truncatus* from Jordan to experimental infection with Egyptian, Ghanian and Nigerian strains of *Schistosoma haematobium,* indicated that they are susceptible to these three strains.

Susceptibility or compatibility are terms referring to the ability of miracidia of a certain strain of a parasite to penetrate the potential vector snail and develop to further larval stages in its intermediate host tissue. Many factors may play important roles in determining whether snail population is susceptible or not to a certain strain of the parasite. Some of these considerations are: geographical origin of the parasite and the snail host, physical factors including temperature, light, oxygen tension and the pH of the water and other physiological, biochemical and immunological factors that may affect the development of the larval stages of the parasite in the snail intermediate host (Wajdi, 1964; Wajdi et al., 1979; Wright, 1962).

The susceptibility of various populations of *Bulinus* snails to the Nigerian strain of *S. haematobium* has been studied by various investigators; Cowper (1954) obtained positive results when he exposed the subspecies *B. t. rohlfsi* from Nigeria to a local strain of the parasite from Nigeria, while Wright (1962) obtained negative results when he exposed the same Nigerian subspecies to another local strain of *S. haematobium* also from Nigeria. Webbe and James (1971) indicated that Iranian *B. truncatus* was susceptible to the Nigerian schistosome parasite, while Wright (1962) reported that other *Bulinus* snails from Angola, Egypt, Ghana, Malagasy and Mauritania were

refractory to infection with a *S. haematobium* strain from Nigeria.

Our studies represent the first attempt to expose *B. truncatus* snails from Jordan to the Nigerian strain of *S. haematobium.* Under laboratory conditions at which the studies were conducted, all 5 populations proved to be susceptible. The infection rates among the populations ranged from 22.8% (Mahrab Ahmed) to 34.8% (Zour Al-Hamam), and statistical analysis of the data obtained, indicated no significant differences in the infection rates among the population studied ($0.1 < p < 0.25$). These similar infection rates may be due to the use of a strain isolated previously from Nigeria and maintained in the laboratory by passage through hamsters for more than 8 years. Also, *B. truncatus* snails from Jordan have probably never been exposed naturally to any strain of *S. haematobium* and therefore may have the potential to be infected more readily with various strains of the parasite.

For the Ghanaian strain of *S. haematobium,* specificity of the local strains of the schistosome parasite to their local snail vector was demonstrated by McCullough (1959) and Paperna (1968). Wright (1962) indicated that *B. t. rohlfsi* snails from Nigeria and Angola were refractory to infection with the Ghanaian parasite, while the Gambian *B. globosus* was susceptible to infection with the Ghanaian *S. haematobium* despite the geographical location of these countries to Ghana. It is probable that Wright (1962) may have used a *B. globosus* borne strain of the Ghanaian parasite.

All the five Jordanian populations of *B. truncatus* were susceptible to the Ghanaian strain of the schistosome parasite. The infection rates ranged from 18.9% (Zarka River) to 28.1% (Zour Al-Hamam). No significant differences were observed among the five Jordanian *B. truncatus* ($0.75 < p < 0.90$).

The susceptibility of *B. truncatus* from Jordan to infection with the Egyptian strain of *S. haematobium* has been reported by Saliba et al. (1981). They exposed two populations of *B. truncatus* to an Egyptian strain of *S. haematobium* which has been maintained in the laboratory through passage in hamsters for 23 years and they obtained infection rates which ranged from 14 to 92% using different doses of miracidia. Ayed and Saliba (1985) found no significance in infection rates when they exposed each of the 5 populations of the vector snail to the Egyptian strain of the parasite collected from many infected Egyptian workers residing in Jordan. The results obtained from the present study supports those of Ayed and Saliba (1985), since no significant differences in infection rates were observed in the present study even though infection rates ranged from 7.5% (Zour Al-Hamam) to 35% (Zarka River) when exposed to 5 miracidia/snail (individually) and from 29% (Zour Al-Hamam) to 52.4% (Mahrab Ahmed) when exposed to 10 miracidia/snail (en masse).

Variation in susceptibility of different populations of *B. truncatus* from Egypt to other Egyptian strains of *S. haematobium* were reported by several workers (Lo, 1972; Frandsen, 1979; Mohamed, 1982), in which they interpreted such variations to the assumption that there is more than local one strain of the parasite in certain areas, which reflects variations in the infectivity among snail populations. On the other hand, Egyptian strain of the parasite proved to be adapted to several *Bulinus* snail vectors; it was infective to *Bulinus* snails from Congo, Ethiopia, Gambia, Iran, Iraq, Jordan, Libya, Mauritania, Morocco, Palestine, Rhodesia, Sudan, South Africa, Tanzania, West Aden and Yemen (Gismann, 1954; Le Roux, 1954; Witenberg and Saltinik, 1957; Lo, 1972, Araffa et al., 1973; Frandsen, 1979a; Wajdi et al., 1979; Saliba et al., 1981; Ayed and Saliba, 1985).

It seems that *B. truncatus* snails from Jordan have the ability to acquire infection with a variety of Egyptian strains of *S. haematobium.* This is especially true in view of the results reported by Saliba et al. (1981) who used a 23 year old laboratory maintained Egyptian strain, and Ayed and Saliba (1985) who probably used many strains of the parasite when they exposed 5 *B. truncatus* populations,

since the eggs were collected from many infected Egyptian workers residing in Jordan, who came from different regions of Egypt. The strain used in the present study was isolated in 1981 from Cairo area and has been maintained in the laboratory for years.

It is concluded that the infection rates obtained and the fact that all populations were susceptible to the three strains tested indicate that the Jordanian *Bulinus* have the characteristics which may be homogenous as regards their ability to become infected with various geographic strains of *S. haematobium.* Further studies concerning this aspect of the study should be continued. Also, the results obtained herein, imply that more attention should be taken to prevent the establishment of transmission foci of the disease in Jordan. This statement is made in view of the fact that many thousands of foreign laborers originating from schistosomiasis endemic areas in neighboring countries came to Jordan each year seeking employment especially in agricultural areas located in close proximity to snail vector infested areas.

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