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Further Studies on the Relationship between *Bulinus truncatus* from Jordan and *Schistosoma haematobium* from Egypt

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Introduction

Until recently, the threat of schistosomiasis to Jordaninans was thought to be minimal or non-existent due to absence of the snail intermediate hosts required for transmission of the disease and the dearth of autochthonous infections. Small scale surveys conducted prior to 1975 on selected water bodies failed to detect snail species capable of supporting the larval development of human schistosomes (Abdel Azim and Gismann, 1956; Chu, 1969). However, in 1975 and thereafter, *Bulinus truncatus*, an important snail host for *Schistosoma haematobium*, has been recorded from several locations in Jordan. The snail was first found in the Muthalath Al-Masri area in the Jordan Valley in 1975 by Saliba *et al.* (1976) then in Jarash in 1978 (Saliba and Salameh, 1981). Additional foci were subsequently detected in the reservoir of the King Talal Dam, Sheikh Hussein, Zour Al-Hamam and Tal Salman springs in the Jordan Valley and in the Zarqa River (Saliba *et al.*, in preparation). The presence of *B. truncatus* in Jordan in conjunction with thousands of foreign nationals suffering from urinary schistosomiasis (mainly Egyptians) increases the potential for the establishment and spread of schistosomiasis in Jordan (Saliba *et al.*, 1980).

The primary objectives of the present study were to evaluate and compare the susceptibility of Jordanian *B. truncatus* populations from numerous foci to infection with *S. haematobium* obtained from infected Egyptian nationals working in Jordan. Previous studies on the susceptibility of *B. truncatus* from two foci in Jordan yielded conflicting results. Saliba *et al.* (1981) recorded moderate to high susceptibility while Daoud (1982) reported low susceptibility for one group and total resistance to infection for another. The present work shows conclusively that Jordanian *B. truncatus* from all sites tested is highly susceptible to *S. haematobium* from Egypt and capable of supporting the parasite to the infective stage.

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Materials and Methods

Source of the parasite

Schistosoma haematobium eggs used in the present study were obtained from infected Egyptians working in the Jordan Valley during 1982. The specimens were collected with the help of teams from the Malaria and Schistosomiasis Section of the Ministry of Health.

Sources of snails

The Jordanian populations of *B. truncatus* used were reared from snails originally collected by the second author from five different sites in the country (Fig. 1) as outlined below.

1) The Muthalath Al-Masri snail population (JM) originated from specimens collected in 1976 from a cemented reservoir in the Muthalath Al-Masri area approximately 13 km. south of Deir Alla Village in the Jordan

Valley.

2) The Jarash population (JJ) was originally collected in 1978 from an ancient Roman pool near the town of Jarash.

3) The King Talal Dam population (JD) was reared from snails collected at various sites in the reservoir of the King Talal Dam on numerous occasions since 1980.

4) The Sheikh Hussein population (JH) was reared from specimens collected in 1981 from Sheikh Hussein spring in the northern part of the Jordan Valley.

5) The Zour Al-Hamam population (JZ) originated from snails collected at Zour Al-Hamam spring in the northern part of the Jordan Valley in 1981.

The Egyptian population of *B. truncatus* used in the present study was obtained from the University of Lowell in 1980. This snail population was originally collected in Egypt in 1964 and has been maintained at the University of Michigan at Ann Arbor since that time.

Maintenance of snails

Cultivation and maintenance of stock and experimental snail populations were done as recommended by Liang (1974). The average snail room temperature during the study period ranged from 25.5 to 27.5°C.

Hatching of *S. haematobium* eggs

Urine samples were brought to the laboratory within 48 hours after collection from patients. Urine was centrifuged at 1100×g for 10 minutes then decanted. The pellet containing the eggs was diluted with filtered snail-conditioned water (SCW), recentrifuged and the second pellet was then poured into small Petri dishes and diluted again with SCW for hatching of eggs. A lamp (75 Watt) was placed about 50 cm above the dishes to enhance hatching.

Snail exposure to *S. haematobium* miracidia

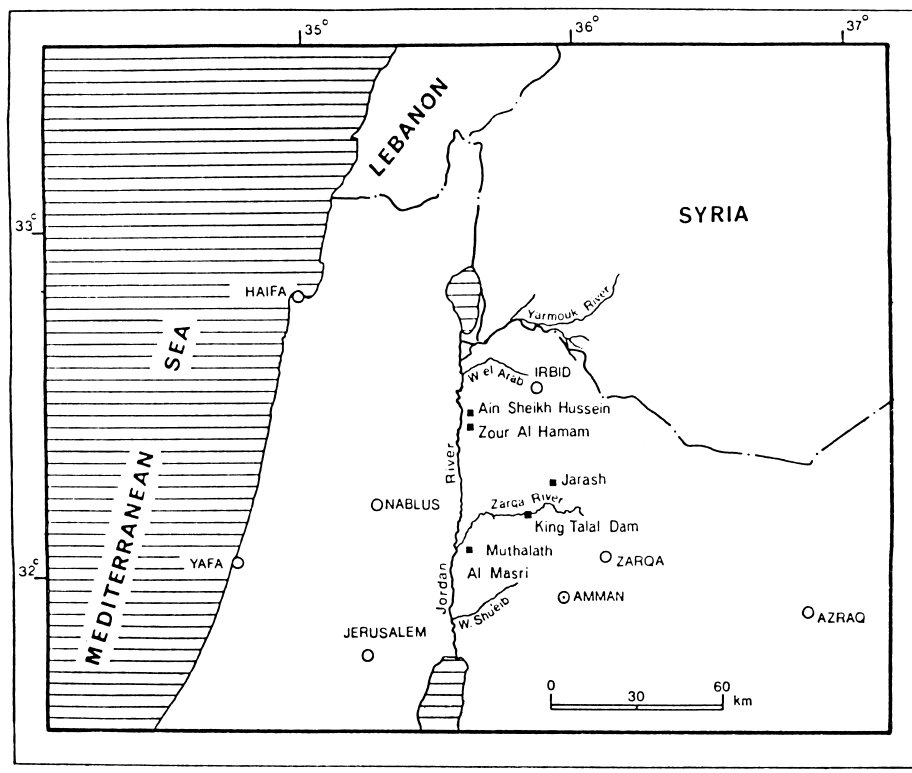

Fig. 1 Sites in Jordan from which *Bulinus truncatus* snails were collected.

Snails were individually exposed to 4-6 miracidia in glass vials (5×1.5 cm). Miracidia were pipetted from Petri dishes into vials within 1-2 hours of hatching. This transfer contributed 2-2.5 ml of water into each vial. Snails of 1-2 mm in shell length were then added to the vials and a lamp (75 Watt) was placed about 50 cm above the vials to stimulate miracidial activity. Exposure of snails to miracidia was terminated after 3-3.5 hours of exposure by transfering the snails from the vials into Petri dishes where they were subsequently maintained. Each dish contained a mud mound and cultures of a species of the blue green alga *Nostoc*. Control snails for the survival studies were similarly maintained.

Monitoring of infection

Thirty days after exposure, the snails were individually placed in vials containing 2 ml of filtered, aerated tap water. A lamp was placed 50 cm above the vials to stimulate emergence of cercariae. Positive snails were checked daily for survival. Negative snails were checked daily for a period of one month after the first shedding of cercariae by positive snails. Snails remaining negative at that time and those that had died during the prepatent period were dissected and checked for cryptic infection with early larval stages of the parasite.

Statistical analysis

Differences in the infection and survival rates of snails were considered significant if the Z values were more than 1.96 at the 5% significance level. When means were tested for significance, the t-test was used and differences were considered significant if (P) was equal to or less than 0.05.

Results

Infection rate

The average infection rates for the Jordanian *B. truncatus* populations ranged from 45% for JJ to 60% for JD snails (Table 1). Differences in susceptibility among the five Jordanian snail populations were not statistically significant (Z-values ranged from 0.045 to 1.570). The infection rate for the Egyptian snails was 19%, significantly lower ($Z \geqq 2.27$) than for the snails from Jordan.

Prepatency survival rates

The average survival rates for the Jordanian snail populations after exposure to infection ranged from 46 to 67%. Only the difference between the survival rates of the JZ and JH populations was significant ($Z=2.20$). Survival rates of the control snails ranged from 41 to 88%. There were significant differences between the survival rates of the exposed and control snails of JM ($Z=3.58$) and JH ($Z=3.59$) populations only. Egyptian *B. truncatus* exposed to infection showed a survival rate of 55% compared to 50% for their controls.

Table 1 Average infection and survival rates of *B. truncatus* snails from Jordan and Egypt exposed to infection with *S. haematobium* from Egypt

Snail Population	Snails		
	Exposed* No.	Surviving %	Infected† %
JJ			
Exposed	95	52	45
Control	45	47	—
JM			
Exposed	63	54	50
Control	40	88	—
JD			
Exposed	113	53	60
Control	46	41	—
JH			
Exposed	63	46	55
Control	42	81	—
JZ			
Exposed	48	67	59
Control	40	73	—
Egypt			
Exposed	49	55	19
Control	40	50	—

* Compiled from several experiments
† No. of infected/no. of surviving

Post patency survival

Average and maximum post patency periods for infected, exposed uninfected, and control groups of the 5 Jordanian populations of *B. truncatus* snails are shown in Table 2. The average post patency periods for the 3 groups ranged from 14 to 60, 24 to 213 days, respectively. In general, the infected snails survived for significantly shorter periods than their corresponding controls ($p<0.01$). Similarly, infected snails lived for significantly shorter periods than the exposed uninfected snails of JJ and JM populations ($p<0.01$). The average post patency period for the three infected Egyptian snails was only 10 days; much lower than the periods for the corresponding exposed uninfected and control snails.

Length of prepatent period and duration of infection

The period between exposure of snails to miracidia and the first emergence of cercariae (prepatent period) ranged from 38 to 40 days in all of the populations studied under the employed rearing conditions. Infected snails continued to shed cercariae until death and no spontaneous recovery or self-cure was observed. The degree and pattern of cercarial

Table 2 Survival of infected, exposed uninfected and control snails of the different populations of *B. truncatus* snails

Snail Population	Snail Status	No. of Snails Observed	Average Post-patency (days)	Differences of Averages	
				t-value	P
JJ	Infected	15	17(20)*	9.60	P<0.01
	Uninfected	10	90(100)	0.51	0.6<P<0.7
	Control	13	96(120)		
	Infected-Control			11.43	P<0.01
JM	Infected	10	53(85)	13.46	P<0.01
	Uninfected	15	213(300)	4.35	P<0.01
	Control	12	130(190)		
	Infected-Control			3.47	P<0.01
JD	Infected	25	60(150)	1.30	0.2<P<0.3
	Uninfected	14	73(129)	2.32	0.02<P<0.05
	Control	14	112(145)		
	Infected-Control			3.86	P<0.01
JH	Infected	13	14(30)	1.99	0.05<P<0.1
	Uninfected	25	24(52)	1.37	0.1<P<0.2
	Control	14	32(46)		
	Infected-Control			3.48	P<0.01
JZ	Infected	17	51(128)	1.86	0.05<P<0.1
	Uninfected	10	79(154)	0.53	0.6<P<0.7
	Control	15	88(120)		
	Infected-Control			2.82	P<0.01
Egypt	Infected	3	10(11)	3.40	P<0.01
	Uninfected	11	68(105)	0.49	0.6<P<0.7
	Control	12	74(90)		
	Infected-Control			4.21	P<0.01

* Numbers in parentheses represent the days when the last snails died.

production by infected Jordanian *B. truncatus* snails through their post patent period have not yet been studied. However, preliminary observations on the number of cercariae shed within an 8-hr period [9:00-18:00] by infected Jordanian snails 2-5 weeks post patency showed that they could shed from 40 to 100 cercariae per snail per 8 hours.

Discussion

Infection rates

The five Jordanian populations of *B. truncatus* tested were highly susceptible to infection with *S. haematobium* from Egypt. Infection rates ranged from 45% (JJ) to 60% (JD). These rates were significantly higher ($Z \geq 2.27$) than the infection rate observed for Egyptian *B. truncatus* (19%). The infection rates of *B. truncatus* populations from Jordan with *S. haematobium* from Egypt are comparable to rates reported by many workers in other countries. Witenberg and Saliternik (1957) and Wajdi *et al.* (1979) exposed *B. truncatus* from Palestine and Iraq respectively, to *S. haematobium* from Egypt and both reported 30% infection rates. Lo (1972) found that 20% of *B. truncatus* from Iran became infected with *S. haematobium* from Egypt while Frandsen (1979) observed infection rates of 50 to 62% for the same intermediate host/parasite relationship. Frandsen (loc. cit.) also reported a 22% infection rate for *B. truncatus* from Yemen exposed to Egyptian *S. haematobium*.

The high susceptibility (50%) of the JM population reported here is comparable to the 57% infection rate observed by Saliba *et al.* (1981), for the same snail population. It is interesting to note that such similar rates of infection were obtained in spite of differences in the source of the parasite (human vs. hamster) and the size of the exposed snails (1-2 mm vs. 3-4 mm). The present findings and those of Saliba *et al.* (1981) are in marked contrast to the very low (1.5%) infection rate encountered by Daoud (1982) for the same snail population. Likewise, the 45% infection rate reported here for the JJ population is higher than the 14% rate reported by Saliba *et al.* (1981) and the zero infection rate observed by Daoud (1982) for the same snail population. It is possible that the differences between the present findings and those obtained by the workers cited resulted from procedural differences between the studies. For example Daoud obtained eggs from a single patient rather than pooling eggs from many patients and she tested larger snails (3-4 mm). The high susceptibility of JM and JJ snails observed in this work, as contrasted to the two previous studies, suggests that it may be important to use miracidia from pooled urine samples from numerous patients or even from patients from different localities for the evalution of snail susceptibility to infection with schistosomes. Similar suggestions have also been proposed by Frandsen (1979) and Mohamad (1982).

The susceptibility of Egyptian *B. truncatus* reported here (19%) was much lower than that of the Jordanian snails exposed to the same pool of miracidia and also lower than the 50% infection rate recorded earlier by Saliba *et al.* (1981) for the same Egyptian snail population. It is difficult to compare the low infection rate of Egyptian *B. truncatus* obtained in this study with the results of Saliba *et al.* (loc. cit.) since the snails used in the two studies were of different sizes (1-2 mm vs. 3-4 mm) and the source of the parasite used also varied (human vs. hamster). Nevertheless, the present findings suggest that the Jordanian *B. truncatus* snails are at least as susceptible to *S. haematobium* from Egypt as are Egyptian snails.

Differences in susceptibility of *B. truncatus* from Egypt to Egyptian *S. haematobium* have also been encountered by other authors. Moore *et al.* (1953) and Lo (1972) reported infection rates of 50 and 3%, respectively, for various populations of *B. truncatus* from Egypt exposed to different geographic strains of the parasite. Frandsen (1979) also found that the infection rate of different populations of *B. truncatus* from Egypt with Egyptian strains of *S. haematobium* varied, ranging from 33 to 49%.

Survival rates during pre- and post patency

In the present study, approximately 50% of the Egyptian and Jordanian *B. truncatus* populations exposed to *S. haematobium* miracidia survived the prepatent period. Furthermore, the survival rates of the corresponding controls were comparable ($Z \leq 1.37$) with those of the exposed snails except for JM and JH control snails which had significantly higher survival rates ($Z = 3.58$). One factor that could have contributed to the rather high mortalities seen in both exposed and control snails is the size of the snails used (1 to 2 mm shell length). Snails in this size range are very young and more fragile than older, larger snails used by other workers. Several studies have shown that the average prepatent survival time of snails is affected by their age at the time of exposure to miracidia. Moore *et al.* (1953) reported a mortality rate of 46% in one-day old snails, 21% in one-week old snails and 20% in 1.5 to five-week old snails. Chu *et al.* (1966a) found that one week old snails had a mortality rate of 39% compared to 24% for snails five to six weeks old.

Differences are evident in the prepatent survival rates for JM and JJ snails obtained in this work compared to those reported by Saliba *et al.* (1981) and Daoud (1982) who also worked with these two populations. In the present study, the survival rate for JM snails was 54% and that for JJ snails was 52%, whereas the survival rates for the corresponding controls were 88 and 47% respectively. Saliba *et al.* (1981) found that the prepatent survival rates for JM and JJ snails exposed to 10 miracidia of *S. haematobium* were 77 and 93%, respectively, while Daoud (1982) reported survival rates of 72% for JM snails and 43% for JJ snails compared to 100% survival rates for their controls. In the two cited studies, however, larger snails (3–4 mm shell length) were used.

High prepatent mortality rates have also been recorded in other populations of *B. truncatus*. Malek (1958) reported a 30% mortality rate in *B. truncatus* from Sudan exposed to a Sudanese strain of *S. haematobium* and Webbe and James (1972) found a 41% mortality rate for four- to six-week old *B. truncatus* from Iran exposed to an Iranian strain of the parasite. More recently, Frandsen (1979) reported a 37% mortality rate in Libyan *B. truncatus* exposed to *S. haematobium* from Egypt but snails from Ghana, Morocco, Egypt and the Sudan had very low mortality rates when exposed to the same Egyptian strain of the parasite.

In the present work, the post patent periods of infected snails were significantly lower than those of the controls of all populations studied and the exposed uninfected JJ, JM and Egyptian snails ($p < 0.01$). Furthermore, the post patent periods of infected JD, JH and JZ snails were also lower than in the corresponding exposed uninfected snails where the difference in longevity was either significant (JD ; $0.02 < p < 0.05$) or closely approaching the significance level (JH and JZ ; $0.05 < p < 0.1$). In JM, JD and JZ populations, the average post patent longevity of infected snails exceeded 1.5 months, much longer than the infected Egyptian *B. truncatus*. Furthermore, a few infected Jordanian snails survived for more than 100 days post patency, The significance of such survival periods in disease maintenance and transmission becomes more evident when the number of cercariae shed by one infected snail during its post patent period is considered.

The average post patent longevity of *B. truncatus* reported in this study is somewhat shorter than others have reported. Chu *et al.* (1966a) recorded an average post patency of 107 days in young snails from Iran exposed to an Iranian strain of *S. haematobium*, while the maximum life span of a cercariae-positive snail was 329 days. The prepatent period ranged from 31–56 days. Chu *et al.* (1966b) also recorded a maximum life span of 170 days in Iranian snails exposed to Iranian *S. haematobium* while the average survival time of infected snails was 71 days. Frandsen (1979) recorded average post patent periods of 54 and 63 days with maxima of 72 and 65 days for two strains of *B. truncatus* from Egypt with an Egyptian strain of the parasite. However, much higher post patent periods ($X = 124$ days and maximum $= 186$ days) were reported by

Frandsen (1979) for infected *B. truncatus* from Morocco, Iran and Libya. It is difficult to determine the cause of differences in the post patent periods observed. Numerous experimental differences including the strain of the snail and the parasite, the protocol used to infect snails, age of exposed snails, and rearing techniques complicate comparison.

Length of prepatent period and duration of infection.

The length of the prepatent period of infected snails ranged from 38 to 40 days. Similar prepatent periods for both Egyptian and Iraqi snails infected with Egyptian strains of the parasite have been reported (Moore *et al.*, 1953; Wajdi *et al.*, 1979). A prepatent period between 36 to 42 days was recorded by Saliba *et al.* (1981). However, Webbe and James (1972) reported a prepatent period of 30-45 days in Iranian *B. truncatus* exposed to *S. haematobium* and Frandsen (1979) observed a prepatent period of 26 days in several strains of *B. truncatus* which were compatible with miracidia from Egypt. Several factors affect the length of the prepatent period including strain of snails (Frandsen, 1979) and temperature at which snails are maintained (Pflüger *et al.*, 1984).

In the present study, snail infections persisted as long as the snails lived. This is in agreement with the results of the above-mentioned authors except for Webbe and James (1972) who reported a self-cure in a few specimens of *B. truncatus* from Iran.

From the findings of this study, it is apparent that the level of compatability between Jordanian *B. truncatus* and *S. haematobium* from Egypt is comparable to or higher than that of Egyptian *B. truncatus*. In particular, the following findings support this conclusion: 1) there was a significantly higher rate of infection in Jordanian snails than in Egyptian snails and 2) both the pre- and post patent survival periods of the Jordanian and Egyptian snails were similar in length. The results therefore confirm earlier comments by Saliba *et al.* (1980) to the extent that schistosomiasis haematobia constitutes a serious threat to Jordan. Efforts by the Ministry of Health and other agencies to prevent the establishment of the disease should be maintained and strengthened.

Summary

Bulinus truncatus snails, originally collected from five sites in Jordan, were found to be highly susceptible to experimental infection with *Schistosoma haematobium* from Egyptian laborers in Jordan. Snails from Jarash (JJ) had the lowest infection rate (45%) with a survival rate until patency of 52%, whereas snails from the King Talal Dam (JD) had the highest infection rate (60%) with a survival rate of 53%. Reference Egyptian snails had a significantly lower infection rate (19%) than the Jordanian snails with a survival rate of 55%. The average post patent survival of infected Jordanian snails was significantly less than that of either the exposed uninfected or the control snails in most of the populations studied. It ranged from 14 days for Sheikh Hussein spring (JH) to 60 days for JD snail populations with maximal survival periods that ranged form 20 to 150 days for JJ and JD snails, respectively. All infected snails continued to shed cercariae until death.

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ヨルダンの貝 *Bulinus trumcatus* とエジプトのビルハルツ住血吸虫との宿主関係に関する研究

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ヨルダン国内の五つの地域すなわち，Muthalath Al-Masri (JM)，Jarash (JJ)，King Talal Dam (JD)，Sheikh Hussein (JH) および Zour Al-Hamam (JZ) 由来の貝 *Bulinus truncatus* は，ヨルダンにいるエジプト人労働者から得られたビルハルツ住血吸虫ミラシジウムの感染に対して，いずれも感受性が高いことが判明した．感染率は45% (JJ)～66% (JD) でありエジプトの同種の貝の感染率19%に比し有意に高かった．セルカリア遊出までの感染貝の生存率は，52% (JJ)，53% (JD)，55% (エジプトの貝) であり，両者ともほぼ同じであった．セルカリア遊出後の感染貝の生存率は，大部分の貝で，非感染対照群に比し，有意に低かった．生存日数は14日 (JH)～60日 (JD) であり，最長生存日数は20日 (JJ)～150日 (JD) であった．感染貝はすべて，死に至るまでセルカリアを遊出し続けた．