Journal of the Egyptian Society of Parasitology, Vol.43, No.2, August 2013
J. Egypt. Soc. Parasitol., 43(2), 315– 325

SCHISTOSOMA MANSONI CERCARIAL HOST LOCATION AND INFECTION UNDER SIMULATED NATURAL CONDITIONS IN EGYPT

By

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Abstract

This study was performed in water ditches under simulated natural conditions in Egypt to elucidate the effect of various environmental factors on *Schistosoma mansoni* cercarial host location and infection of the definitive host (using albino mice). Evaluation of these factors was dependent on both infection rate of exposed mice as well as the schistosome worm load under the same experimental conditions. The seasonal water temperature proved to be a very important factor and this was proven by the infection rate of mice and the worm load recovered were lower in January and April (16^{o}C and 22^{o}C midday water temperature) and much higher in July and October (29^{o}C and 25^{o}C). The daytime factor is similarly important as temperature illustrated by the schistosome infection of mice groups exposed at 8-10 am was much higher than in groups exposed between 1pm and 3pm ($p<0.001$). The greatest infection rate of mice and worm load were obtained when the shedding snails were close to the exposed group of mice. Both criteria increased with the increase of cercarial density in the water. The length of exposure period is also an extremely important factor for schistosome infection, being highest 87.5% ($p<0.001$) in3 hours exposure period. Infection rate was found to be 88.2% and 55.6% of shedding snails were located at water surface and midway to the bottom, respectively, and no infection occurred when located at the bottom. The schistosome infection of mice decreased in presence of increasing density of the floating plant *Eichhornia crassipes* in the ditch water, but low condensation of the submersed plant *Ceratophyllum demersum* appeared to have stimulating effect.

Key words: Egypt, *Biomphalaria alexandrina, Schistosoma mansoni*, *Mus musculus*

Introduction

Schistosomiasis has been, and is still known to be complicated unsolved health problem encountering medical, socio-economic and behavioral parameters (Abou-El-Naga and Radwan, 2012**)**. It was essential to understand factors that control the transmission of schistosomiasis in order to prevent its outbreaks, as well as to provide feasible control measures (Curtale *et al*, 2010). The severity of risk factors for the acquisition of schistosomiasis depends basically on the concentration of cercariae population in natural water bodies at a certain time. This concentration is the result of total production of cercariae from infected snails and is

mainly correlated with the environmental conditions under which the snail population exists (Ibrahim *et al*, 2005; Sayed *et al*, 2004; King, 2001; Squires, 2000; Yousif *et al,* 1998 a &b). Therefore, the study of cercarial population in natural water bodies should provide a useful mean for locating schistosome transmission foci and help in evaluating the success of control programs.

In this study exposing albino mice to *S. mansoni* cercariae in natural water was used under simulated standardized conditions, to study physical and biological factors controlling schistosome transmission as exhausted from rate of mice infection and worm load. Results obtained should contribute to better understanding of the epidemiology of the disease and consequently better planning of its molluscicidal and the chemotherapeutic control.

Materials and Methods

This work was carried out in the Snail Research Station of Theodor Bilharz Research Institute (TBRI) **(**Fig.1) which was first described by Yousif *et al*. (1992). The experiments were performed in ditches with muddy bottom and sloping banks, 30 m long, 150 cm wide at water level, and 50 cm wide at the bottom and supplied with underground water. The major abiotic and biotic components of the ditches water (fauna and flora) were checked and found almost similar to natural irrigation ditches. *Schistosoma mansoni* cercarial host location and infection were studied under several environmental conditions namely seasonal water temperature, pattern of diurnal cercarial shedding from snails, light and shade, distance between host (mice) and shedding snails, cercarial density in water, cercarial age, exposure period, location of shedding snails in the ditches and type and density of vegetation present. Cercariae-shedding *Biomphalaria alexandrina* (Ehrenberg) and albino mice *Mus musculus* CD1 strain were obtained from the Schistosome Biological Supply Center (SBSC) at TBRI.

Otherwise stated, 30 infected snails were placed in plastic mesh cages as a source of cercariae and hanged in each ditch at 10 cm below water surface.

The mice were exposed using a special device for tail immersion (Fig. 2) (Yousif *et al,* 1996a). The mice exposure device was floated in such a way that all tails were completely immersed in the water. Six mice were exposed in each ditch and three replicates were used in each case. Water temperature during exposure period was 22-26°C except when studying the seasonal water temperature. The exposure period was 3hr. after which the mice were removed from the restraining chambers and maintained in cages in the laboratory for 6 - 8 weeks except the desired exposure period when studying the effect of exposure period. The mice were then sacrificed and schistosome worms were collected by perfusion from the portal and mesenteric veins. The whole livers were also squashed between two glass plates and examined for worms using a stereo-microscope.

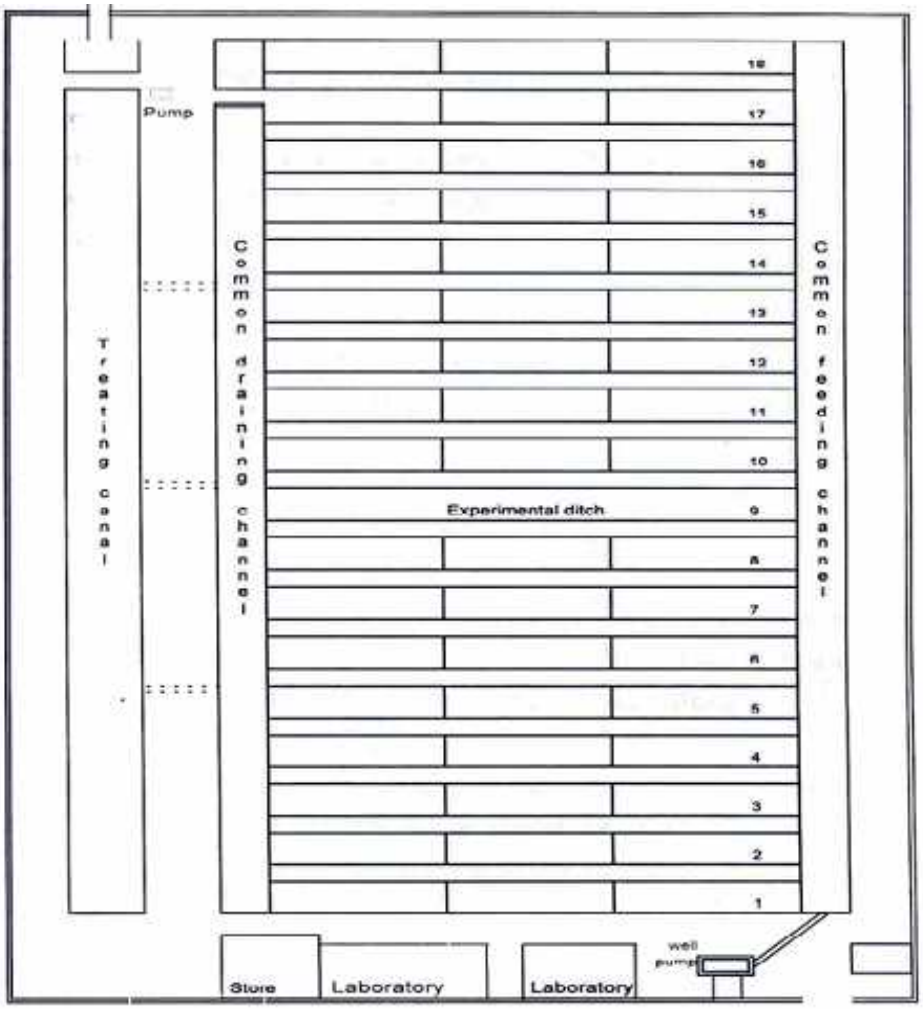

Fig. 1: Plan of experimental area 1:300 (After Yousif *et al*, 1992).

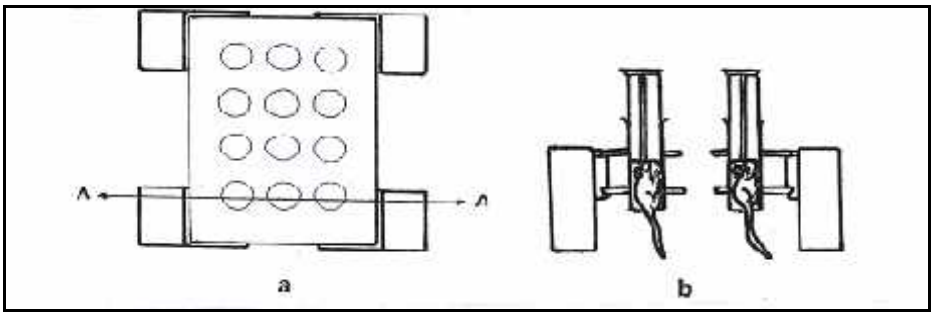

Fig. 2: Mouse immersion apparatus (top view) showing layout of restrainers (a) and longitudinal section A-A showing individual mouse restrainers (b) (After Yousif *et al*, 1996a).

Statistical analysis: Data was computerized and infection rates of groups were compared by chi-square values of contingency tables (Southwood, 1978). Worm load/mouse groups were compared by *t*-test (Clopton and Janovy, 1990).

Results

Seasonal water temperature: Four degrees of water temperature at midday representing various seasons of the year were tested, namely 16±1°C (January), 22±1°C (April), 29±1°C (July), and 25±1°C (October). There was considerably low infection rate of mice in January and April (Tab.1) without significant difference but with significant difference ($p<0.05$) in worm load. In October and July, the infection rate was much higher than previous seasons ($p<0.001$) without significant difference but with highly significant difference ($p<0.01$) between October and July in worm load.

Table 1: Effect of seasonal water temperature on *S. mansoni* infected mice under simulated natural conditions.

Temperature at exposure	Mice number at exposure	Surviving mice number at sacrificing	Infected mice number	% infection	Worm load/mouse	
					Mean	±S.D.
Jan. 16±1	36*	30	8	26.7	2.7	1.2
Apr. 22±1	18**	15	5	33.3	5.5	3.4
Oct. 25±1	36*	26	22	84.6	5.9	3.6
Jul. 29±1	18**	15	15	100	13.6	6.9

* 6 replicates, 6 mice each, ** 3 replicates, 6 mice each

Pattern of diurnal snail shedding: For studying the effect of day time on host location and infection, mice were exposed on hourly schedule to water containing the shedding snails in the experimental ditches (from 8am-4pm). The shedding snails were transferred each hour to other ditch and a new group of mice was exposed simultaneously with shed-ding snails, without significant difference (Tab. 2) between schistosome infected mice groups exposed at 8-9am and 9-10am. Infection rate significantly decreased ($p<0.05$) later and mice exposed at 3-4pm were not infected. Worm load/mouse increased significantly from 8-9am to 9-10am ($p<0.001$), mean worm load decreased from 10-11am till the experimental end at 4pm.

Table 2: Effect of pattern of diurnal snail shedding on *S. mansoni* infected mice under natural conditions.

Exposure time (hrs.)	Surviving mice number at sacrificing*	Infected mice number	% infection	Worm load/mouse	
				Mean	±S.D.
8-9 am	17	15	88.2	6.3	2.5
9-10	14	12	85.7	23.9	25.4
10-11	13	8	61.5	6.1	7.5
11-12	15	8	53.3	3.6	3.1
12-1 pm	14	4	28.6	2	0.8
1-2	14	1	7.1	2	0
2-3	15	1	6.7	2	0
3-4	16	0	0	0	0

* 3 replicates, 6 mice each.

Light and shade: Two groups of mice were exposed to shedding snails in ditch water, one under normal day light and the other under shade. Both groups of mice (Tab. 3) had got schistosome infection with no significant difference in infection rate and in worm load/ mouse.

Table 3: Effect of light and shade on infection of mice with *S. mansoni* under simulated natural conditions.

Treatment	Surviving mice number at sacrificing*	Infected mice number	% infection	Worm load/mouse	
				Mean	±S.D.
Light	15	14	93.3	9.9	8.5
Shade	15	12	80.0	9.3	6.3

* 3 replicates, 6 mice each.

Distance between mice and shedding snails: Both infection rate (Fig. 3) and worm load of mice decreased considerably by increasing distance between shedding snails and mice. The greatest infection was obtained when the shedding snails were close to mice. There was significant decrease ($p<0.01$) in the two parameters with increase of distance. Both decreased rapidly within one meter and remained almost constant after that till 5m.

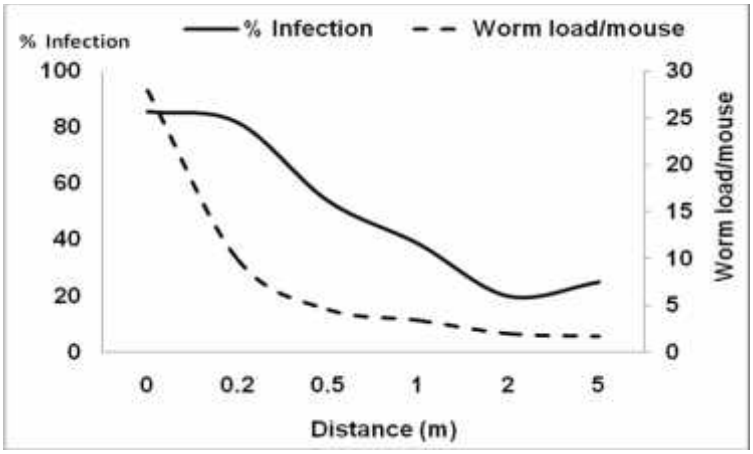

Fig. 3: Effect of distance between mice and shedding snails on mice infection with *S. mansoni* natural conditions.

Cercarial density: Several numbers of cercariae (100-5000) were introduced into separate ditches at similar points. With relatively low (Tab. 4) cercarial densities (100-700 cercariae/ditch) none or very low mice infection but increased rapidly later. Cercarial age: This experiment evaluated the effect of cercarial age on mice infection. The cages with equal numbers of snails were covered with foil sheets in separate ditches after sunset. The next day the sheets were removed and snails were exposed to light for an hour (9am-10am) in all ditches and then removed. One group of mice was exposed to wa-

ter in one ditch from 10am - 11am i.e. to cercariae < 2 h old. Other groups were successively exposed in other ditches from 10.00h- 12.00h, 1200-14.00, 14.00-16.00 and from 16.00-18.00 h i.e. to cercariae <3 h, <5h, <7h and <9 hours old, respectively. There was no significant difference (Tab. 5) in infection rate with 3 h old cercariae but with a significant decrease ($p<0.05$) in worm load between cercariae <3h and <2h old. With further increase of cercarial age, a significant reduction in both infection rate and worm load was observed.

Table 4: Effect of cercarial density on infection of mice with *S. mansoni* under natural conditions.

Number of cercariae /ditch.	Surviving mice number at sacrificing*	Infected mice number	% infection	Worm load/mouse	
				Mean	±S.D.
100	14	0	0	0	0
200	17	1	5.9	1	0
300	15	1	6.7	1	0
500	15	0	0	0	0
700	14	1	7.1	6	0
1000	18	3	16.7	3.3	1.2
2000	18	14	77.8*	5.2*	4.4
5000	18	15	83.3*	10.3*	7.02

*($p<0.001$) 3 replicates, 6 mice each.

Table 5: Effect of cercarial age on infection of mice with *S. mansoni* under natural conditions.

Age of Cercariae (hour)	Surviving mice number at sacrificing*	Infected mice number	% infection	Worm load/mouse	
				Mean	±S.D.
<2	16	10	62.5	3.5	2.01
<3	15	9	60	2.2	1.5
<5	17	3	17.6	1.3	0.6
<7	12	2	16.7	1	0
<9	14	2	14.3	1	0

* 3 replicates, 6 mice each.

Exposure period: Highly significant increase ($p<0.001$) in infection rate and worm load between exposure for 2 hrs. than for an hr. or less (Tab. 6), without significant difference ($p>0.05$) in infection between 2 & 3hr. exposure period.

Table 6: Effect of exposure period on mice infection with *S. mansoni* under natural conditions.

Exposure to contaminated water (hours)	Surviving mice number at sacrificing*	Infected mice number	% infection	Worm load/mouse	
				Mean	±S.D.
0.25	14	9	64.3	4.0	3.1
0.5	12	6	50.0	4.2	3.3
1	15	8	53.3	4.3	2.4
2	13	11	84.6	5.6	4.7
3	16	14	87.5	7.6	7.8

* 3 replicates, 6 mice each.

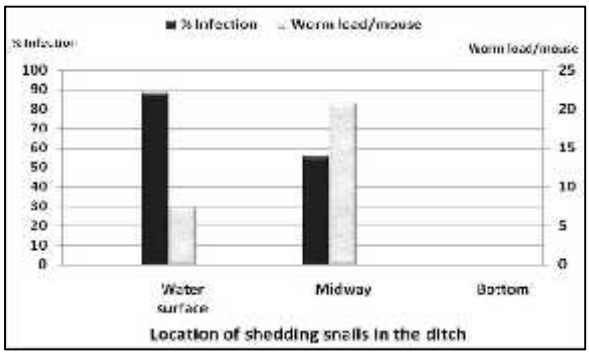

Fig. 4: Effect of location of shedding snails on water body on mice infection with *S. mansoni* under simulated natural condition

Location of shedding snails in water body: Three locations of shedding snails in the ditch were tested, namely near water surface (10 cm below water surface), at mid-way to the bottom (25 cm below water surface) and at the bottom (50 cm from water surface). The infection rate (Fig. 4) was 88.2% & 55.6% at water surface and midway to the bottom, respectively, without infection when the shedding snails were located at the bottom. Mean worm load/ mouse was much larger when the snails were located at midway than at water surface with a highly significant difference ($p<0.01$).

Type and density of vegetation: the floating plant *E. crassipes* and the submerged one *C. demersum* were obtained from natural water courses and transferred to the laboratory where they were thoroughly washed by running water and cleaned from snails, eggs, insects and filamentous algae before introduced to experimental ditches. Two densities of each plant were used; a density which covers the whole water surface and half that density. Schistosome infection of mice (Fig. 5) decreased (infection rate and worm load/ mouse) with increasing of *E. crassipes* in the ditch. In the case of *C. demersum* (Tab. 7) low plant condensation had stimulant effect on cercarial infection of mice i.e. highly significant increase in infection rate and mean worm load/mouse ($p>0.01$) in comparison with control, without significant difference between high condensation and control ($p>0.05$).

Table 7: Effect of type and density of vegetation on mice infection with *Schistosoma mansoni* under simulated natural conditions

Condensation	Surviving mice at sacrificing*	No. infected	% infection	Worm /mouse		%
				Mean	±S.D.	Change
Eichhornia crassipes						
Control without plant	11	11	100	6.6	5.6	0
Half	12	11	91.7	4.7	2.2	-8.3
High	9	7	77.8	11.7	8.7	-22.2
Ceratophyllum demersum						
Control without plant	14	10	71.4	5.9	4.7	0
Half	14	13	92.9	14.2	8.5	+30.1
High	15	12	80	7.8	4.9	+12.04

* 3 replicates, 6 mice each.

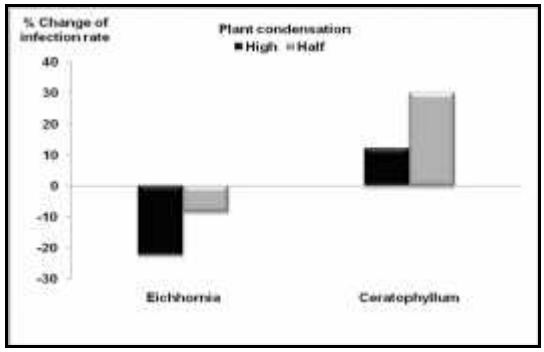

Fig. 5: Effect of type and density of vegetation on mice infection with *S. mansoni* under natural conditions.

Discussion

Schistosomiasis is the second most prevalent worldwide parasite next to malaria. It has significant economic and public health consequences in many developing countries (Engels *et al*, 2002). The present results showed that increasing of water temperature was associated with increasing of infection rate of mice. These data agreed with many authors (Stirewalt and Fregeau, 1965; Purnell, 1966a, b; Christensen *et al,* 1979). They found that penetration of *S. mansoni* cercariae in mice occurred at temperatures from 7^{o}C to 45^{o}C. Below and above these degrees the ability of cercariae to penetration was reduced. Also, agreed with Yousif *et al.* (1996 a, b) who found that mice exposure was seriously affected by water temperature.

Information about the diurnal snail shedding may be important because of the risk of infection from any single exposure varies with the time of day in which water contact occurs. These results partially confirm previous data of some investigators. Asch (1972) found the peak of emergence of *S. mansoni* cercariae was between 10am & 12 pm. Kloos *et al(*1982) found that the daily cercariae sampling showed highest densities between 0700 and 0900 am. In the Nile River, they found that all cercariae were encountered in the morning. A peak of cercariae density was between 13:30 & 15:30 pm (Prentice and Ouma, 1984).

The present results showed that, both groups of mice exposed in light and in shade had got schistosome infection and there was no significant difference in infection rate between them. However, these results are not in accordance with Yousif *et al*. (1996b) who found that the number of cercariae was highest under light, while it decreased in shade.

The mice infection rate decreased with increase of distance between mice and shedding snails in natural ditches. The infection rates were high in groups of mice hanged at or near the cercarial release point and dropped dramatically at 5 meters from this point. These results agreed with Sandt (1973), Cai *et al (*1999) who claimed that the mean

number of recovered worms decreased gradually as mice were far from the site of cercarial release. However, Jordan *et al.* (1980) claimed that water current may carry infective cercariae up to as long distance in natural habitats.

Increasing cercarial concentration and exposure period led to increase of infection rate and worm load of mice. But increasing of cercarial age led to decreasing of infection rate in mice, being 62.5% and 14.3% after 2 and 8 hours, respectively. These findings agreed with Stirewalt and Fregeau (1965) who found that best penetration of *S. mansoni* cercariae into mice under laboratory conditions was obtained with 50 cercariae per exposure for at least one hour. They found that decreasing of cercarial concentration lead to reduction of worms recovered. The present results proved that duration of cercarial exposure is an important factor in infection of exposed mice. Christensen *et al.* (1979) found that no increase was observed in the number of penetrated worms after 90 and 120min. exposure. Conversely, Blumenthal and Jewsbury (1983) showed that increasing of cercarial age (2-10 hours) without effect on percentage worm recovery.

It is clear from the present findings that there is a highly significant difference in mice infection depending on location of shedding snails in ditch water and there was no infection when snails were located at the bottom. Warren and Peters (1967) observed that *S. mansoni* cercariae move upwards towards the surface of the water. Ibrahim *et al* 2005 *et al* reported that the location of snails has an important effect on the distribution of cercariae and the largest numbers of cercariae were located at the surface of the water than were obtained from midway and bottom of habitat.

The present results show that Infection of mice decreased significantly with increasing of *E. crassipes* in the ditch. In low *C. demersum* condensation there was stimulating effect on cercarial infection of mice. There was highly significant difference in infection rate and mean worm load/mouse ($p>0.01$) in comparison with control. These findings agreed with Klumpp and Chu (1980) who confirmed that the aquatic macrophyte *Ceratophyllum* is the most important ecological factor for sustaining high levels of cercarial transmission of *S. haematobium* and increasing density of *Ceratophyllum* was correlated with increasing levels of cercarial transmission potential in the water contact sites as well as infection in the village populations. Ibrahim *et al* (2005) in Ghana reported that the mean number of cercariae recovered from areas of low plant density was higher than the number recovered from the areas with high plants density.

Acknowledgment

The authors heartily dedicated this work to the late Dr. Hind El-Gindy, Professor of Zoology, Faculty of Science, Cairo University, Egypt.

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