Bull. Org. mond. Santé
Bull. Wld Hlth Org. } 1966, **34**, 131-133

Host-Parasite Relationship of *Bulinus truncatus* and *Schistosoma haematobium* in Iran

3. Effect of Water Temperature on the Ability of Miracidia to Infect Snails

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Laboratory studies were made in Iran to test the effect of water temperature on the ability of the miracidia of S. haematobium *to penetrate* B. truncatus. *Snails three to four weeks old were exposed to two miracidia each for two hours at nine water temperatures ranging from 10°C to 38°C. After exposure, all the snails were kept in aquaria at room temperature. The cercaria-positive rates of these nine groups of snails showed that the optimum exposure temperature was in the range 20°C-30°C. The infection rate was low at temperatures outside this range. It is concluded that the optimum transmission seasons in bilharziasis-endemic areas in Iran are spring and autumn but that transmission still occurs in hot summer and cold winter months although to a much smaller extent.*

In combating bilharziasis, investigators are usually interested in determining the transmission season of the cercaria-to-man phase of the parasite cycle. As far as the epidemiology of the disease is concerned, however, the penetration of a miracidium into a snail is as important as the penetration of a cercaria into man. However, study of the miracidium-to-snail phase has generally been neglected, especially in *Schistosoma haematobium*.

In studying the miracidium-to-snail phase, the effect of the environmental water temperature should first be considered. Brumpt & Werblunsky (1928) reported that in Corsica the optimum temperature for exposing the miracidia of *S. haematobium* to *Bulinus contortus* is 24°C-25°C. For the sake of comparison, it was thought to be of interest to restudy the problem in Iran, using the local strain of *S. haematobium* and the local intermediate host, *B. truncatus*.

Gaud, Arfaa & Zeini (1962) reported that the diurnal water temperature of snail habitats in the endemic area of Iran ranged from 8°C to 26°C in January 1961 and 26°C to 38°C in August 1961. In 1963, when the present experiments were performed, the water temperature in the summer also varied from 26°C to 38°C, but in January 1964 the diurnal water temperature ranged from 0°C to 18°C. Since miracidia are very inactive at temperatures below 10°C, a water temperature ranging from 10°C to 38°C was used for the present study.

MATERIALS AND METHODS

Laboratory-bred *Bulinus truncatus* three to four weeks old and of similar size were divided into nine groups of 45-150 each, according to the number of available snails. Miracidia were obtained from eggs originating from the pooled urine of bilharziasis patients in the endemic area. Two miracidia each were placed in test-tubes containing 0.5 ml of unchlorinated tap-water, and all the test-tubes to be dipped into a water-bath at a particular temperature were placed in a rack. The water-bath was set for the temperature scheduled for the experimental exposure. Snails, contained in a beaker of water, were placed in the water-bath 30 minutes before exposure, and the rack of test-tubes containing miracidia was placed in the water-bath at least 10 minutes before exposure. Each of the snails in the experimental group was then placed in one of the test-tubes. The exposure time was two hours. Altogether nine water-baths were used, the temperatures being 10°C, 12°C, 14°C, 15°C, 20°C, 25°C, 30°C, 35°C and 38°C.

After two hours' exposure, the snails were taken out of the test-tubes and washed several times in

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INFECTION RATES OF SPECIMENS OF *B. TRUNCATUS* EXPOSED TO MIRACIDIA OF *S. HAEMATOBIUM* FOR TWO HOURS AT VARIOUS TEMPERATURES

Water temperature (°C)	Number of exposed snails	Number of snails alive on the 50th day after exposure	Number of cercaria-positive snails			Percentage of cercaria-positive snails
			Found by shedding prior to 50th day after exposure	Found by crushing on the 50th day after exposure	Total	
10	100	85	1	2	3	3.5
12	50	40	1	2	3	7.5
14	50	42	3	4	7	16.7
15	100	90	9	16	25	27.8
20	100	80	24	21	45	56.2
25	150	118	55	16	71	60.2
30	97	87	30	19	49	56.3
35	97	89	33	5	38	42.7
38	45	37	12	0	12	32.4

unchlorinated water. They were then kept in laboratory aquaria at room temperature, 21°C-24°C. Thirty-five days after exposure, the surviving snails in each group were tested individually in test-tubes for cercarial shedding. Snails that failed to shed cercariae by the 50th day after exposure were crushed. Those that were found to contain cercariae were added to the number of cercaria-positive snails.

The infection rate of the snails in each experimental group was obtained by dividing the number of cercaria-positive snails by the number of surviving snails and multiplying the quotient by 100.

RESULTS

The infection rates for snails exposed to two miracidia at the various temperatures used are given in the accompanying table. The optimum temperature range for a high rate of infection is clearly 20°C-30°C. Although the rate of infection of snails exposed at 25°C was higher than that of the snails exposed at 20°C or 30°C, statistical tests showed that the difference was not significant at the 5% level. However, the rate of infection of snails exposed at 20°C is significantly different from that of snails exposed at less than 20°C. Similarly, the rate of infection of snails exposed at 30°C is significantly different from that of snails exposed at more than 30°C.

The table also reveals that the infections of snails exposed at 20°C or above were mostly found by cercaria-shedding prior to the 50th day after exposure, but that the infections of snails exposed at 15°C or below were mostly found by snail-crushing on the 50th day after exposure. This result appears to indicate that the exposure temperature had some effect on the development of *S. haematobium* in *B. truncatus*.

DISCUSSION

Our findings concerning the optimum temperature range for infection of 20°C to 30°C are similar to those of Brumpt & Werblunsky (1928) for *B. contortus* exposed to the miracidia of *S. haematobium* in Corsica. However, DeWitt (1955) found that when *Australorbis glabratus* was exposed to the miracidia of *S. mansoni*, the infection rate rose with temperature up to 35°C.

In the cold season of Iran—late December, January, and early February—the maximum water temperature is about 20°C and the minimum 10°C or below. At a temperature of 8°C, eggs of *S. haematobium* can survive for eight days, and miracidia from eggs exposed for less than three days to this temperature will not lose their invasive power (authors' unpublished data). Although the infection rate of snails exposed to miracidia at temperatures of 10°C-20°C is low, it is necessary to take account

of the fact that the longevity of miracidia in cold water is increased. It is reasonable to believe that snails can still acquire infection in Iran's cold season.

In the hot season—late June, July and August—the water temperature in the bilharziasis endemic areas in Iran usually ranges from 30°C to 38°C during the day. At such temperatures, the infectivity of miracidia to snails is low, and the snail population itself is greatly reduced. The summer months are obviously not a good season for the infection of snails. For this reason, the transmission of the parasites to snails occurs mostly in certain special snail habitats such as a cold spring, a borrow-pit fed constantly with canal water, or a shaded pool in which the water temperature does not exceed 30°C.

We may conclude that, as far as the water temperature of the snail habitat in Iran is concerned, a transmission potential from the miracidia of *S. haematobium* to *B. truncatus* may exist all the year round, but spring and autumn will be the optimum seasons.

ACKNOWLEDGEMENTS

The authors express their appreciation to Dr C. Mofidi, Director of the Institute of Public Health Research, for his permission to publish this paper; to Dr H. Bijan, Assistant Director of the Institute, Dr F. Arfaa, Dr S. Darugar and the staff of the Dezful station for their co-operation; and to Mr A. Roushanee and Mr M. Shahbazei for their technical assistance.

RÉSUMÉ

Ce troisième article d'une série de quatre sur les relations hôte/parasite entre *Bulinus truncatus* et *Schistosoma haematobium* étudie l'influence de la température sur la pénétration du miracidium chez le mollusque en utilisant des souches locales de l'un et de l'autre. Des mollusques âgés de 3-4 semaines ont été exposés chacun à deux miracidiums dans des tubes à essai individuels maintenus deux heures à une température constante. Neuf groupes ont subi chacun une température différente comprise entre 10°C et 38°C. Après cette exposition, les mollusques ont été lavés et conservés dans des aquariums à la température du laboratoire: 21°C-24°C. Les survivants de chaque groupe ont été examinés 35 jours après cette exposition pour noter l'existence d'une émission cercarienne. Les mollusques n'ayant pas encore émis de cercaires le 50e jour ont été broyés et considérés comme infectés s'ils renfermaient des cercaires. Le taux d'infection de chaque groupe a été obtenu en divisant le nombre de mollusques positifs par le nombre de survivants et en multipliant le quotient par 100. Les taux d'infection les plus élevés ont été observés dans les groupes exposés aux températures comprises entre 20 et 30°C; ils étaient bas en dehors de ces limites. Les mollusques ayant émis des cercaires avant le 50e jour appartenaient aux groupes exposés à 20°C et plus.

Les auteurs concluent que les saisons optimales de transmission de la bilharziose dans les zones endémiques de l'Iran sont le printemps et l'automne; la transmission peut s'effectuer au cours des mois chauds d'été et des mois froids d'hiver mais elle est alors de bien moindre importance.

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