

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

4. Effect of Month of Infection on Cercarial-Incubation Periods of *S. haematobium* and *S. bovis*

K. Y. CHU,¹ J. MASSOUD² & H. SABBAGHIAN²

Studies were conducted each month for one year to determine the cercarial-incubation periods of Schistosoma haematobium and Schistosoma bovis in Bulinus truncatus for different months of infection. The snails were kept in outdoor aquaria in order to simulate the natural temperature conditions in the endemic bilharziasis areas of Iran.

The results showed that the cercarial-incubation periods of these two schistosome species varied with the environmental water temperature. Snails exposed in August had the shortest incubation period, and snails exposed in November the longest. The cercarial-incubation period for S. haematobium was longer than that for S. bovis in all months. The difference between the cercarial-incubation period of these two species varied from three to 18 days, being greater in the winter than in the summer.

It has been concluded that the low temperature of the water in the winter retards the development of miracidia into cercariae and that the winter is therefore the poorest season for potential transmission. In summer, in spite of the hot weather, snails may still shed cercariae, but spring and autumn are the optimum seasons for cercariae transmission.

In order to formulate efficient control measures for bilharziasis, a study of the monthly infection rates of snails in the endemic areas to determine the cercarial-transmission season is one of the first requirements. It was thought to be useful to carry out such a study on both *Schistosoma haematobium* and *Schistosoma bovis*, since both these species infect *Bulinus truncatus* in Iran. To differentiate the cercariae of the two species of schistosome is laborious; there is no easy and practical method that is suitable for use in the field. Hence an indirect method for determining the potential cercarial-transmission seasons of the two species by maintaining infected snails in outdoor aquaria was attempted, and the results of our studies are reported in this paper.

MATERIALS AND METHODS

Twenty outdoor cylindrical concrete aquaria 1 metre in diameter and 30 cm deep were built out-

side our laboratory in Dezful, Iran. Garden soil was used as a substrate. After the aquaria had been filled with water, their sides became covered with a layer of algae in a few days. Bean stems and leaves were used for snail food in the summer; fresh lettuce was used in other seasons. The water was changed once a week in the summer and about once in two or three weeks in other seasons. Maximum and minimum temperatures were recorded daily except during holidays. The diurnal water temperatures of the natural habitats of the snails were used as an index for regulating the water temperature in the aquaria by adjusting the water level of the latter.

The eggs of *S. haematobium* were collected from the pooled urine of patients in the endemic areas. The eggs of *S. bovis* were obtained from the livers of laboratory-infected *Tatera indica*, a rodent species. *Schistosoma bovis* had previously been maintained in the laboratory by infecting *B. truncatus* (with miracidia obtained from the eggs of *S. bovis* originating from a naturally infected cow) and by subsequently infecting *Tatera indica* with cercariae from *B. truncatus*.

¹ WHO Malacologist in Iran.

² Institute of Public Health Research, Teheran, Iran.

TABLE 1
MONTHLY WATER TEMPERATURES OF THE AQUARIA

Month	Water temperature (°C)			
	Mean minimum temperature	Range of minimum temperatures	Mean maximum temperature	Range of maximum temperatures
June 1963	24.6	23-26	31.9	30-35
July	26.4	25-29	33.8	32-37
August	26.5	25-29	35.0	33-38
September	25.3	21-29	31.1	26-39
October	22.2	19-25	27.3	23-30
November	17.7	15-22	21.9	17-27
December	11.2	6-15	17.1	12-24
January 1964	7.6	2-11	14.3	10-18
February	9.9	7-14	19.4	12-24
March	14.9	13-18	25.9	15-32
April	17.4	16-19	28.6	26-33
May	19.0	17-25	31.7	30-34

Laboratory-bred snails four to six weeks old were exposed, in groups of five, to 10-20 miracidia in dish seals for six hours. After the exposure, the snails were placed in the outdoor aquaria. The exposure of snails to miracidia of *S. haematobium* and *S. bovis* was performed on the same day. Single exposures of this kind were made for different groups of snails in successive months from June 1963 to May 1964.

Tests for cercaria-shedding by the exposed snails were started at the following times: for exposures to miracidia made during the period June to October, from the 13th to the 16th day after exposure; for exposures during the period November to February, from the 40th to the 60th day after exposure; for the exposure in March, on the 30th day after the exposure; for the exposure in April, on the 25th day after the exposure; and for the exposure in May, on the 19th day after the exposure.

Cercaria-shedding tests were made in the laboratory daily or twice weekly during cold months by placing the snails singly in a test-tube under artificial light for three hours. For each group, the minimum and maximum cercarial-incubation periods of the

TABLE 2
CERCARIAL-INCUBATION PERIODS OF *S. HAEMATOBIMUM* IN *B. TRUNCATUS* RESULTING FROM EXPOSURE TO MIRACIDIA IN DIFFERENT MONTHS

Month when snails exposed to miracidia	Number of snails exposed	Number of snails alive at end of maximum cercarial-incubation period	Number of snails shedding cercariae	Cercarial-incubation period (days)		
				Minimum	Maximum	Difference
June	50	26	14	24	28	4
July	30	24	16	21	23	2
August	30	15	9	19	24	5
September	50	33	12	21	28	7
October	30	16	8	36	50	14
November	48	10	7	146	154	8
December	60	18	9	130	146	16
January	50	21	14	94	104	10
February	50	30	17	74	80	6
March	40	29	18	50	57	7
April	40	22	16	42	48	6
May	30	17	7	25	28	3

TABLE 3
CERCARIAL-INCUBATION PERIODS OF *S. BOVIS* IN *B. TRUNCATUS* RESULTING FROM EXPOSURE TO MIRACIDIA IN DIFFERENT MONTHS

Month when snails exposed to miracidia	Number of snails exposed	Number of snails alive at end of maximum cercarial-incubation period	Number of snails shedding cercariae	Cercarial-incubation period (days)		
				Minimum	Maximum	Difference
June	20	20	5	21	25	4
July	30	21	18	15	15	0
August	30	25	20	15	17	2
September ^a	—	—	—	—	—	—
October	45	39	22	19	28	9
November	80	30	21	133	141	8
December	30	19	3	115	123	8
January	58	23	17	83	91	8
February	50	50	35	56	61	5
March	40	33	21	38	45	7
April	50	16	8	33	40	7
May	20	12	5	22	22	0

^a Readings not carried out.

TABLE 4
COMPARISON OF THE MINIMUM CERCARIAL-INCUBATION PERIODS FOR *S. HAEMATOBIIUM* AND *S. BOVIS* IN *B. TRUNCATUS* RESULTING FROM EXPOSURE TO MIRACIDIA IN DIFFERENT MONTHS

Month when snails exposed to miracidia	Minimum cercarial-incubation period (days)		
	<i>S. haematobium</i>	<i>S. bovis</i>	Difference
June	24	21	3
July	21	15	6
August	19	15	4
September ^a	21	—	—
October	36	19	17
November	146	133	13
December	130	115	15
January	94	83	11
February	74	56	18
March	50	38	12
April	42	33	9
May	25	22	3

^a Cercarial-incubation period of *S. bovis* not determined in September.

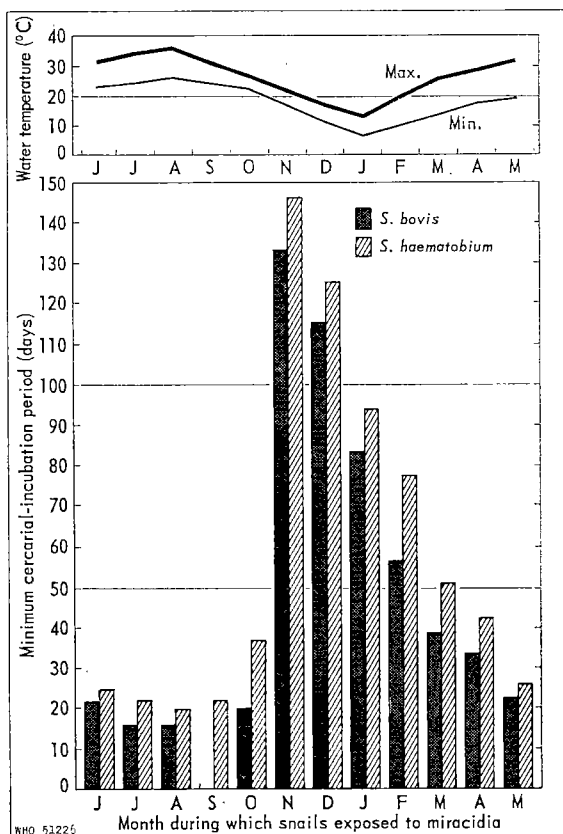
exposed snails were recorded. Comparison of the cercarial-incubation periods among snails in different groups was made according to the minimum periods, unless otherwise specified.

RESULTS

The monthly water temperatures in the aquaria in which the exposed snails were kept are listed in Table 1. The cercarial-incubation periods of *S. haematobium* and *S. bovis* in *B. truncatus* resulting from the successive monthly exposures of the different groups are given in Tables 2 and 3. The differences between the minimum cercarial-incubation periods of the two schistosome species in different months are given in Table 4 and the accompanying figure, which also shows the monthly variations in temperature.

An analysis of the data shows that the cercarial-incubation periods of the two schistosome species varies with the environmental water temperature. The snails exposed in August had the shortest incubation period; the snails exposed in November, the longest period. The cercarial-incubation period of *S. bovis* was shorter than that of *S. haematobium* in all months. The difference between the minimum

MINIMUM CERCARIAL-INCUBATION PERIODS
OF *S. HAEMATOBIIUM* AND *S. BOVIS* IN *B. TRUNCATUS*
IN RELATION TO AVERAGE MONTHLY WATER
TEMPERATURE



cercarial-incubation periods of these two species varied from three to 18 days, being greater in winter than in summer. The difference between the minimum and maximum cercarial-incubation period of each species was less in summer than in winter.

DISCUSSION

Gordon, Davy & Peaston (1934) reported that the cercarial-incubation period of *S. haematobium* in *Physopsis globosa* in Sierra Leone was 22-23 days at a water temperature of 32°C-33°C, 36 days at 26°C-28°C, and 66-68 days at 20°C-22°C. Lengy (1962) found that the cercarial-incubation period of *S. bovis* in *B. truncatus* in Israel was 21 days at a temperature of 28°C. Although the results of our

present findings did not correspond exactly to these previous findings, the trend was the same.

Due consideration must be given to the fact that the development of cercariae in the intermediate host will be influenced by the age of the snails during exposure and by the exposure dosage of miracidia (Chu, Massoud & Sabbaghian, 1966a; Chu, Sabbaghian & Massoud 1966).¹ In the natural habitat of the snails in the endemic areas, snails of different ages occur in the same locality, and the miracidial exposure dosages may differ, not only in different localities but also at different times. Experimental results from the laboratory usually represent a trend but may not correspond well to conditions in the field. Nevertheless, since the present experiments were carried out in environmental conditions simulating the natural ones, we may assume that similar conditions may exist in the local endemic areas.

The fact that snails exposed to the miracidia of *S. haematobium* on 14 October started to shed cercariae on 19 November, 36 days after exposure, and that the last snail of this batch shed cercariae on 3 December, 50 days after exposure, indicates that the water temperature in October and November did not interfere with the development of the cercariae to maturity. However, snails exposed to the miracidia of the same species on 3 November shed cercariae on 28 March, 146 days after exposure, and those exposed in early December, January, and early February started to shed cercariae in early April, about 120, 90 and 60 days after exposure. These figures show that the low temperature in November, December, January and February (especially the latter three months) did retard the development of the miracidia into cercariae. The water temperature ranged from 2°C to 20°C in this period. Similar results were observed in the development of *S. bovis* in *B. truncatus*.

On the basis of the above findings, we may assume that the cercarial-infection season may start in March. Because the cercariae of *S. bovis* usually develop earlier than those of *S. haematobium*, the cercarial-infection season for *S. bovis* possibly begins in the middle of March and that for *S. haematobium* at the end of March. Since the degree of cold weather may vary from year to year, this prediction will accordingly be subject to modification.

¹ See the articles on pages 113 and 121 of this issue.

In winter, we rarely encountered cercariae in snails in the endemic areas. However, it appears from the following experiment that young sporocysts may survive as long as the snails are alive. Snails were exposed to the miracidia of *S. haematobium* at room temperature and five days later were placed in water at 8°C for one day, then cooled to freezing-point and left at 0°C-1°C for four hours. This treatment was repeated on two further days, and the snails were then put back into an aquarium at room temperature. The percentage of snails that later shed cercariae was comparable to that of the control snails, which were exposed to miracidia at the same time but not subjected to the freezing treatment.

The temperature in spring and autumn makes these two seasons favourable for cercarial development. In summer, the water temperature in most shallow snail habitats reaches 36°C-39°C in the afternoon. To find out whether schistosome larval stages would tolerate this high temperature, the following two experiments were performed. Snails exposed to the miracidia of *S. bovis* were kept in an

outdoor aquarium at 37°C-38°C for three afternoons, at 36°C-37°C for seven afternoons, and at 35°C-36°C for three afternoons. These snails later shed cercariae. Snails exposed to the miracidia of *S. haematobium* were kept in an outdoor aquarium at 38°C-39°C for three afternoons, at 37°C-38°C for seven afternoons, and at 36°C-37°C for four afternoons. Cercariae were shed by these snails also. These experiments showed that the development of the schistosome larval stage was not impeded by water temperatures as high as 36°C-39°C. In the field, water levels in the snail habitats usually change once a week in the summer, and the low water levels remain for only one or two days. Snail populations can be greatly reduced in these low water levels in the summer months, and the infectivity of miracidia to snails is also low (Chu, Massoud & Sabbaghian, 1966b).¹ However, the surviving snails, if infected, can still shed cercariae, although the number of cercaria-shedding snails is less than that in spring and autumn.

¹ See the article on page 131 of this issue.

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 M. Hamzah and Mr A. Karimy for their technical assistance.

RÉSUMÉ

Les auteurs ont précédemment publié trois articles sur les relations hôte/parasite entre *Bulinus truncatus* et *Schistosoma haematobium*, dans lesquels ils précisaient l'effet de l'âge du mollusque sur le développement du parasite, l'influence du nombre de miracidiums infectants sur la biologie du vecteur et le développement larvaire du parasite, l'influence de la température sur la pénétration du miracidium chez le mollusque. Dans ce quatrième article, ils rapportent les résultats des études qu'ils ont menées chaque mois, de juin 1963 à mai 1964, pour déterminer les périodes d'incubation cercarienne de *S. haematobium* et de *S. bovis* chez *B. truncatus*. Pour réaliser les conditions de températures naturelles dans la zone d'endémie en Iran, vingt cylindres de 1 m de diamètre et profonds de 30 cm ont servi d'aquariums en plein air. Les températures maximales et minimales ont été notées chaque jour, sauf les jours fériés. Des mollusques âgés de 4 à 6 semaines provenant de l'élevage du laboratoire furent exposés le même jour, par groupes de

5, à 10-20 miracidiums pendant six heures puis placés dans les cylindres. Les miracidiums de *S. haematobium* provenaient d'œufs recueillis dans des urines de malades, ceux de *S. bovis* d'œufs recueillis dans le foie de rongeurs, *Tatera indica*, infectés expérimentalement. Les périodes d'incubation cercarienne ont varié avec la température de l'eau. Les mollusques exposés en août eurent la période d'incubation la plus courte, ceux exposés en novembre la plus longue. La période d'incubation cercarienne fut toujours plus longue pour *S. haematobium* que pour *S. bovis*; la différence entre les périodes d'incubation de ces deux espèces, variant de 3 à 18 jours, était plus grande en hiver qu'en été. Les auteurs concluent que les basses températures de l'eau en hiver retardent le développement des cercaires, et que l'hiver est la saison la moins favorable à la transmission. En été, malgré l'élévation de la température, les mollusques peuvent émettre des cercaires mais le printemps et l'automne sont les saisons optimales de transmission de la bilharziose.

REFERENCES

- Chu, K. Y., Massoud, J. & Sabbaghian, H. (1966a) *Bull. Wld Hlth Org.*, **34**, 113-119
Chu, K. Y., Massoud, J. & Sabbaghian, H. (1966b) *Bull. Wld Hlth Org.*, **34**, 131-133
Chu, K. Y., Sabbaghian, H. & Massoud, J. (1966) *Bull. Wld Hlth Org.*, **34**, 121-130
Gordon, R. M., Davy, T. H. & Peaston, H. (1934) *Ann. trop. Med. Parasit.*, **28**, 323
Lengy, J. (1962) *Bull. Res. Coun. Israel, E*, **10**, 1
-