Effect of aestivation of *Biomphalaria pfeifferi* on the survival and infectivity of *Schistosoma mansoni* cercariae

L. I. BADGER and J. P. O. OYERINDE
Department of Medical Microbiology and Parasitology, Lagos State University College of Medicine, P.M.B 21266, Ikeja, Lagos, Nigeria

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ABSTRACT

Schistosoma mansoni cercariae from post-aestivated *Biomphalaria pfeifferi* remain motile for 20 hours after release. Thereafter, their activity decreases with age. The difference in mortality rate of cercariae from aestivated and non-aestivated *B. pfeifferi* studied here proved to be statistically significant ($P<0.05$) within the first 10 hours of the experimental period. Results of the percentage recovery of worms from different mouse organs infected with cercariae from aestivated and non-aestivated snails varied. The two main organs infected were the liver and intestine. In conclusion, the penetration, migration and maturation of cercariae into adult worms were not affected by the aestivation of *B. pfeifferi*.

KEY WORDS: Biomphalaria pfeifferi.
Schistosoma mansoni.

Introduction

Aestivation is a form of dormancy by which some animals are able to live through hot, dry summer months. It is induced by the removal of water[1] and is central to the ecology of many African freshwater molluscs. The desiccation of a habitat has catastrophic effects on the snail population; however, workers[2,3] have shown that freshwater snails can withstand considerable periods of desiccation through the aestivating process.

Intermediate hosts of *Schistosoma mansoni* have also been shown to carry immature infection through the dry season.[4] In laboratory experiments, *Biomphalaria pfeifferi* was shown[5] to carry *S. mansoni* infection through a period of aestivation and be able to release cercariae within 7–14 days post-aestivation, depending on the age of infection prior to aestivation.

Penetration, migration and subsequent development of *S. mansoni* cercariae are influenced by two major factors: the status (e.g., age) of the cercariae at the time of contact with the host, and the physiological condition of the host at the time of contact.[6] Infectivity is determined by the percentage of cercariae that develop into adult worms in the mammalian host.

Infectivity can be influenced by circumstances related to the development of cercariae in the snail, the conditions of the aqueous environment after leaving the snail, and the susceptibility of the definitive host. Generally, the rate of infectivity has been correlated with the age of the host,[7–10] rather than with environmental factors such as radiation[11] and desiccation.

It is postulated that the desiccation of an *S. mansoni* habitat is an environmental factor that could affect the infectivity and survival of cercariae released post-aestivation. This study aims to confirm or refute this hypothesis.

Materials and methods

Adult specimens of laboratory-bred *B. pfeifferi* (measuring 7–8 mm in diameter) were selected from breeding tanks and infected as describe previously.[5] The infected *B. pfeifferi* were divided into three groups. Group 1 consisted of snails induced to aestivate using a fresh miracidial infection for seven days, and subsequently were reactivated. Snails in Group 2 were subjected to desiccation with seven-day-old miracidial infection and aestivated for seven days before reactivation. Snails in Group 3 were infected but not subjected to desiccation (control group).

Upon reactivation, the two experimental groups were returned to separate culture tanks and maintained until the infection in them was 21 days old. At this point, and on a weekly basis thereafter, all three groups of snails were stimulated separately to shed cercariae by exposure to bright light in a beaker containing chlorine-free water. The three different cercariae suspensions obtained were used to infect three groups of mice.

The paddling method, first described by Moore *et al*.,[12] was used to infect the mice. Briefly, this involved allowing the mice to paddle in cercariae-infected water, a process that mirrored the normal mode of infection. Each mouse was transferred to its assigned jar and the required cercariae suspension was added to it. The jars were covered with perforated lids and allowed to stand for four hours to ensure maximum cercarial penetration.

At two, four, six, eight and 10 weeks following exposure to cercariae, 25–30 mice were sacrificed each week by cervical dislocation and their organs were examined. The liver, spleen, intestine and lungs were removed and placed in separate Petri dishes containing normal saline. Worms were teased from each organ and the numbers recovered were expressed as a percentage of the total, and the percentage

Correspondence to: Dr L. I. Badger
Email: lorinaineta@excite.com/lorinaineta@yahoo.com

Table 1. Effect of aestivation of *B. pfeifferi* on the activity of released cercariae.

Age of cercariae (hours)	Cercariae suspension from post-aestivated *B. pfeifferi*				Control			
	Activity score (%)				Activity score (%)			
	A	B	C	D	A	B	C	D
0	96	4	0	0	99	0	1	0*
2	86	5	0	10	98	2	0	1*
4	69	12	5	14	87	3	2	8*
6	67	12	3	17	80	7	4	9*
8	40	24	9	17	60	16	7	13*
10	35	35	6	24	52	28	6	14*
12	17	44	8	31	26	32	5	38
18	14	18	0	68	21	15	0	74
20	10	11	4	76	18	2	3	78
22	8	6	6	81	18	0	0	82
24	4	2	2	81	6	10	0	84

A: active, B: sluggish, C: immobile, D: dead.

*Statistically significant ($P<0.05$).

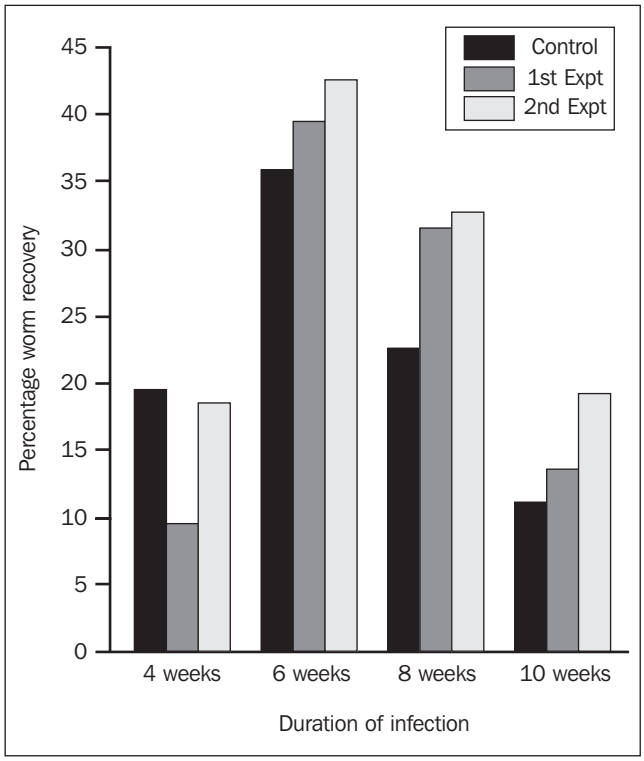

Fig. 1. Percentage recovery of worms from mice exposed to cercariae from post-aestivated snails.

infectivity was also calculated.

Cercarial suspensions obtained from post-aestivation and non-aestivated snails were used to determine survival rates and activity scores. In order to determine cercarial survival rates *in vitro*, 1 mL cercarial suspension from each of the two groups was put into separate 90-mm diameter Petri dishes. The numbers of cercariae found to be actively motile, sluggishly motile, immobile or dead were determined as described previously.[13] Cercarial activity scores are shown in Table 1.

Results

Infection was found predominantly in the liver and the intestine (Tables 2 and 3). In the control group, fewer worms were recovered from the liver at four, six and 10 weeks post-infection, compared with the number recovered from the portal mesentery. The percentages of worms recovered from various organs were variable: for example, more worms were recovered from the liver than from the intestine during the fourth week. Cercarial age pre-infection did not alter the site of worm recovery.

A total of 374 mice were examined in the first and second replicate experiments, while 210 were examined in the control experiments. In all the experiments, worms were recovered four, six, eight and 10 weeks after cercarial exposure (Table 4). Maximum recovery occurred at six weeks post-infection, regardless of whether the cercariae were from aestivated or non-aestivated snails. Figure 1 shows the percentages of worms recovered. Although the difference observed between the first experiment and the control experiment was not statistically significant ($P<0.05$), the difference between the second replicate experiment and the control experiment was significant ($P>0.05$).

Table 1 shows the effect of *B. pfeifferi* aestivation on cercarial survival rate. The difference in cercarial mortality rate from aestivated and non-aestivated *B. pfeifferi* was statistically significant ($P>0.05$) within the first 10 hours of the experimental period. The percentage of actively motile cercariae decreased with time in both experiments (Table 1). Cercarial activity decreased from active to sluggish, and some became immobile prior to death.

Discussion

This study showed that mature cercariae shed by post-aestivated *B. pfeifferi* were as infective as those produced by the unaestivated snails. Similar findings have been reported[14] for the snail *B. glabrata*, which showed that both

Table 2. Percentage of worms recovered from mouse organs infected with cercariae from non-aestivated *B. pfeifferi*.

Age of cercariae (hours)	Site of recovery	Duration of infection (weeks)			
		4	6	8	10
0	Liver	25	8	59	0
	Lungs	0	0	0	0
	Intestine	75	92	41	100
	Spleen	0	0	0	0
4	Liver	16	16	62	0
	Lungs	1	0	0	0
	Intestine	83	84	38	100
	Spleen	0	0	0	0
12	Liver	0	66	55	0
	Lungs	0	0	0	0
	Intestine	100	34	45	100
	Spleen	0	0	0	0
24	Liver	100	67	46	20
	Lungs	0	0	0	0
	Intestine	0	33	54	80
	Spleen	0	0	0	0

Table 3. Percentage of worms recovered from mouse organs infected with cercariae from aestivated *B. pfeifferi*.

Age of cercariae (hours)	Site of recovery	Duration of infection (weeks)			
		4	6	8	10
0	Liver	57	28	47	46
	Lungs	2	0	0	0
	Intestine	41	72	53	54
	Spleen	0	0	56	0
4	Liver	50	52	56	23
	Lungs	7	0	0	0
	Intestine	43	48	44	77
	Spleen	0	0	0	0
12	Liver	54	16	36	38
	Lungs	0	0	0	0
	Intestine	46	74	64	62
	Spleen	0	0	0	0
24	Liver	15	44	33	100
	Lungs	0	0	0	0
	Intestine	50	56	67	0
	Spleen	0	0	0	0

Table 4. Infectivity rates of cercariae from snails aestivated for seven days at different developmental stages.

Group	Total number of mice examined	Mean number of worms recovered			
		4	6	8	10
1*	189	15± 6.24	59± 28.40	45± 23.97	16± 6.63
2†	185	10± 4.53	44± 13.69	29± 10.13	21± 10.3
Control	10	21± 11.75	43± 18.87	36± 9.21	15± 4.26

* Cercariae from snails aestivated for one week with the fresh miracidial infection.

† Cercariae from snails aestivated for one week with seven-day-old miracidial infection.

well-fed and undernourished cercariae were capable of infecting mice and producing adult worms. Furthermore, cercariae from post-aestivated *B. pfeifferi* tended to infect the same organs as those from non-aestivated snails.

However, cercariae from post-aestivated *B. pfeifferi* became less motile more quickly during the first 12 hours after release, which may have been due to a reduction in reserved energy resources. These cercariae either were unable to obtain and conserve sufficient energy from their host after aestivation, or they burned off energy faster than cercariae from unaestivated snails.

One of the ways that cercariae age quickly is by rapid movement, which causes them to lose energy quickly. In the present study, cercariae were maintained in pre-experiment conditions that favoured their survival. They were not subjected to temperature stress or unusual light stimuli, as the water was only agitated when the cercariae were transferred from one container to another. Thus, the early loss of cercarial motility shown by those from post-aestivated snails could not have been caused by stress.

It is known, however, that cercarial lifespan is dependent on food reserves.[14] Post-aestivated snails had gone through a period of starvation during which reserve energy was depleted. Upon reactivation and subsequent maintenance in the laboratory, most (if not all) lost nutrients should have been replenished by the snails before cercarial release commenced; however, comprehensive study of the physiological status of post-aestivated and non-aestivated snails is needed to confirm or refute this.

Autoradiographic tracking studies[15] indicate that nearly all *S. mansoni* cercariae that penetrate mouse skin eventually colonise the lungs. During the present investigation, some mice were sacrificed two weeks after cercarial infection, but no worms were recovered from any other organs examined. It is possible that the schistosomula were very small and might have required a more appropriate extraction technique (eg incorporating autoradiography) for recovery two weeks after infection. Recovery of some worms from the lungs four weeks after infection (Table 2) was a surprise.

Cercarial infectivity declines with the organism's age, probably as a result of the depletion in energy reserves, and it was expected that cercariae from post-aestivated snails would lose their infectivity faster than those obtained from unaestivated snails. However, a combination of *B. pfeifferi* aestivation and the ageing of *S. mansoni* cercariae did not alter percentage infectivity. The cercariae shed by post-

aestivated snails were also capable of producing infection 24 hours after being released.

The difference in percentage worm recovery between the first replicate experiment and the control experiment was not statistically significant, indicating that aestivation had no effect on cercarial infectivity; however, the significant difference observed in the second replicate experiment suggests that results can be variable. □

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