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Effects of temperature on survival, infectivity and *in vitro* encystment of the cercariae of *Echinostoma caproni*

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Abstract

The effects of temperature on survival, infectivity and *in vitro* encystment of *Echinostoma caproni* cercariae in artificial spring water (ASW) were studied. Effects of aging cercariae in ASW at various temperatures showed that at 23°C cercariae achieved 50% survival in 24 h, compared to 92 h at 12°C. Cercariae aged in ASW at 28 and 37.5°C showed 50% survival at 16 and 10 h, respectively. Cercariae aged at different temperatures for various times were used to infect juvenile *Helisoma trivolvis* (Colorado strain) snails maintained in ASW at 23°C. Index of infectivity was based on counting encysted metacercariae in the snails at 8 to 12 h post-infection. Cercariae aged at 23, 28 and 37.5°C showed 50% encystment at 6, 8 and 4 h, respectively. Cercariae aged at 4°C showed 50% encystment in 10 h and cercariae aged at 12°C showed 50% encystment beyond 16 h. Cercariae showed maximal longevity and infectivity in snails when aged at 12°C in ASW. For *E. caproni*, as in other digeneans, the infective period of cercariae is markedly shorter than the maximal life-span at any given temperature. Studies on *in vitro* encystment of *E. caproni* cercariae in Locke's solution:ASW (1:1) showed that encystment was optimal at 23°C (78% encystment) and that it declined to 44% at 28°C and became almost nil (0.02%) at 12 or 37.5°C.

Introduction

Echinostoma caproni is a convenient digenean to study functional biology because it is easy to cycle in the laboratory between *Biomphalaria glabrata* snails and laboratory mice. Therefore, all stages in its life cycle are available for manipulative work. Our laboratory has been concerned with the biology of the free-living cercarial stage of this digenean. Fried *et al.* (2002) examined emergence of cercariae of *E. caproni* from *B. glabrata* under different laboratory conditions and Fried & LaTerra (2002) studied *in vitro* and *in vivo* encystment of the cercariae of *E. caproni*.

Numerous studies have examined longevity and infectivity of various digeneans (see review in Pechenik & Fried, 1995). For most cercariae studied, it is apparent that the infective period of this stage is markedly shorter than the maximum cercarial life span at any given temperature. The relationship between infectivity and survival of *E. caproni* as a function of temperature was examined by Evans (1985). He referred to *E. caproni* as *E. liei* in that study. Preliminary studies in our laboratory noted markedly longer survival times for *E. caproni* cercariae than those reported by Evans (1985). Evans (1985) did not describe the type of water used in his work, i.e. tap water, deionized water (DI), aquarium water, artificial spring water (ASW) or other. Because water quality influences cercarial survival and longevity, we used ASW in our work. In addition to studying cercarial survivial at various temperatures in ASW, we examined infectivity to laboratory raised juvenile *Helisoma trivolvis* (Colorado strain) snails of cercariae aged in ASW at various temperatures from 4 to 37.5°C. This snail is easy to maintain in the laboratory and is a convenient experimental second intermediate host for *E. caproni* cercariae and has been used previously for this purpose (Frazer & Fried, 1998; Fried & LaTerra, 2002).

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The final aspect of this study was concerned with the effects of temperature on *in vitro* encystment of the cercariae of *E. caproni* in a medium of Locke's solution:ASW (1:1). Fried & LaTerra (2002) found effective *in vitro* encystment of cercariae of this species in this medium at 23°C, but the effects of various temperatures on *in vitro* encystment were not studied.

Because the functional biology of *E. caproni* is quite different from that of *E. trivolvis* (see review in Fried & Huffman, 1996), this study will provide new data on the biology of echinostome cercariae in addition to the information that already exists on *E. trivolvis* described by Pechenik & Fried (1995).

Materials and methods

Source of cercariae

Cercariae of *E. caproni* were obtained from *B. glabrata* snails previously infected with miracidia of this echinostome as described by Idris & Fried (1996). Infected snails were used from 7–10 weeks post-infection (PI). To obtain cercariae, snails were isolated individually at room temperature (23°C), in Stender dishes containing 4 ml of ASW prepared as described by Ulmer (1970). The physical and chemical characteristics of ASW were described by Ulmer (1970). About 30 infected snails were used to obtain the several thousand cercariae required for this study.

Effects of temperature on cercarial survival in ASW

Cercariae collected within 1 h post-emergence were transferred to multi-well plates. Each of five wells per plate contained ten cercariae in 0.5 ml of ASW. Each plate was then maintained at either 12, 23, 28 or 37.5°C and cercarial survival was recorded at regular intervals. Cercariae were considered dead when they failed to respond to mechanical stimulation. Each experiment was done three to five times using a total of 150 to 250 cercariae for each temperature.

Effects of temperature on cercarial infectivity in juvenile H. trivolvis *(Colorado strain)*

Snails were removed from the Stender dishes after 30 min to ensure that all cercariae were aged no more than 30 min and no less than 0 min. Cercariae were transferred to multi-well plates, ten cercariae per well in 0.5 ml of ASW, and the plates were maintained at either 4, 12, 23, 28 or 37.5°C. After known time intervals (0.5, 1, 4, 8, 12 or 16 h), the plates were removed from their respective temperatures and placed at 23°C at which time a single *H. trivolvis* juvenile snail (Colorado strain; shell diameter 1–3 mm) was added to each well. From 8 to 12 h post-infection, each snail was removed from the plate, crushed between a glass slide and coverslip, and examined under a compound microscope at 100 × to count the number of cysts formed per snail as described by Fried & LaTerra (2002). The 8 to 12 h PI time criterion was used because Fried & LaTerra (2002) noted that maximum encystment of *E. caproni* in *H. trivolvis* junvenile snails occurred by 6 h postinfection. From three to 12 snails were exposed to cercariae at each temperature. Snails that died prior to their examination for cysts were eliminated from the count.

In vitro *encystment experiments at various temperatures*

In vitro encystment studies were done as described in Fried & LaTerra (2002). To obtain *in vitro* formed cysts, encystment studies were done in 9-cm diameter glass finger bowls, each containing 40 ml of Locke's:ASW (1:1) and maintained at either 12, 23, 28, or 37.5°C. About 75 newly emerged cercariae were transferred to a bowl at each temperature and observations on encystment were made at 48 h. Each experiment was done three to seven times (see table 1) to obtain observations on 211 to 534 cercariae. The number and percent of *in vitro* formed cysts were determined at 48 h.

Results

The effects of temperature on cercarial survival in ASW are summarized in fig. 1. Cercariae reached 50% survival at about 24 h at 23°C compared to 92 h at 12°C. Survival decreased at 28°C and 37.5°C where cercariae reached 50% survival at approximately 16 and 10 h, respectively. Cercariae maintained at 12°C had the longest maximum survival. Survival of cercariae at higher and lower temperatures was significantly different (one way ANOVA, $P < 0.05$) than that at room temperature (23°C) at 20 and 26 h.

The effects of temperature on infectivity are summarized in fig. 2. Cercariae maintained at 4°C reached 50% infectivity at approximately 10 h. Cercariae maintained at 12°C remained infective the longest, maintaining greater than 50% infectivity after aging 16 h at that temperature. Cercariae maintained at 23°C showed 50% infectivity within the range of standard error from 6 to 12 h of age. Those maintained at 28°C reached 50% infectivity at approximately 8 h. Cercariae maintained at 37.5°C showed the shortest infectivity period with 50% infectivity at 4 h and no infectivity at 12 h.

The effects of temperature on *in vitro* encystment are summarized in table 1. The percent encystment *in vitro* was almost 2 × greater at 23 than 28°C, and encystment was practically nil at 37.5 or 12°C.

Discussion

Temperature has a major effect on survival, infectivity, and encystment of cercariae of *E. caproni*. While some studies of the effects of temperature on survival and

Table 1. Effects of temperature on *in vitro* encystment of *Echinostoma caproni*.

Group	Temperature (°C)	Total number of cercariae used	Number (%) of encysted metacercariae
A	12	310	6 (0.02)
B	23	211	165 (78.2)
C	28	534	234 (43.8)
D	37.5	360	7 (0.02)

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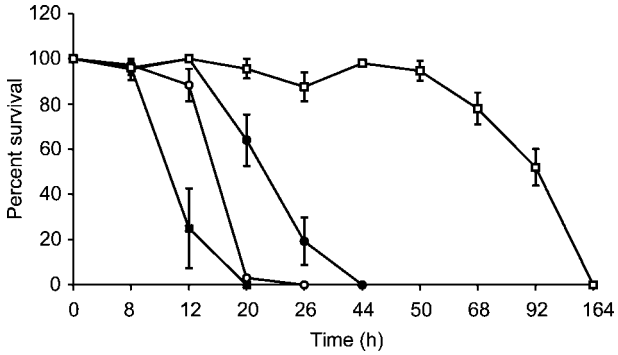

Fig. 1. Effects of temperature on survival (± standard error) of cercariae of *Echinostoma caproni* maintained in ASW. •, 23°C; ■, 37.5°C; ○, 28°C; □, 12°C.

infectivity of *E. caproni* are available (Evans, 1985; Meyrowitsch *et al.*, 1991), their quantitative results do not agree with our findings. Evans (1985) did not report the type of water used in his experiments, making it difficult to explain the marked difference in 50% survival between his study and our study. At 12°C, Evans (1985) reported 50% survival at approximately 45 h, while we found 50% survival at about 92 h at that temperature. The discrepancies at other temperatures were not as marked, ranging from 4 to 6 h longer in our study than those of Evans (1985).

Meyrowitsch *et al.* (1991) used a standard water type of pH 8 to 8.5 (approximately 60% DI water and 40% tap water) containing calcium (40 to 50 ppm). The points of 50% infectivity and maximum infectivity found by Meyrowitsch *et al.* (1991) were lower than the results of our study. Meyrowitsch *et al.* (1991), however, used *B. glabrata* rather than *H. trivolvis* as an experimental second intermediate host and this could in part account for the differences. Meyrowitsch *et al.* (1991) also reported cumulative data from snails restricted to different water levels possibly limiting contact and reducing infectivity, another factor that may account for some of the differences in results.

Despite quantitative differences, the current study agrees with the conclusions of Evans (1985) and Meyrowitsch *et al.* (1991) that cercarial survial and infectivity of *E. caproni* are temperature-dependent and the infective life-span of the cercariae is shorter than the total life-span. In the present study, the time to reach 50% survival at 28°C was 2 × as long as the time to reach 50% infectivity. At 37.5°C, the time to reach 50% survival was 2.5 × longer than the time to reach 50% infectivity.

Another important fact revealed by this study is that cercariae may be maintained at 12°C for about 92 h and still produce 50% infectivity in *H. trivolvis* (Colorado strain) juvenile snails maintained at 23°C. Additionally, 80% infectivity could be maintained for at least 16 h at this temperature. Maintenance at 4°C, however, caused cercariae to only retain 50% infectivity for approximately 10 h. As noted by Evans (1985), *E. caproni* cercariae were unable to infect the *B. glabrata* second intermediate host at 12°C, but his study did not include infectivity at room temperature after cercarial maintenance at various temperatures.

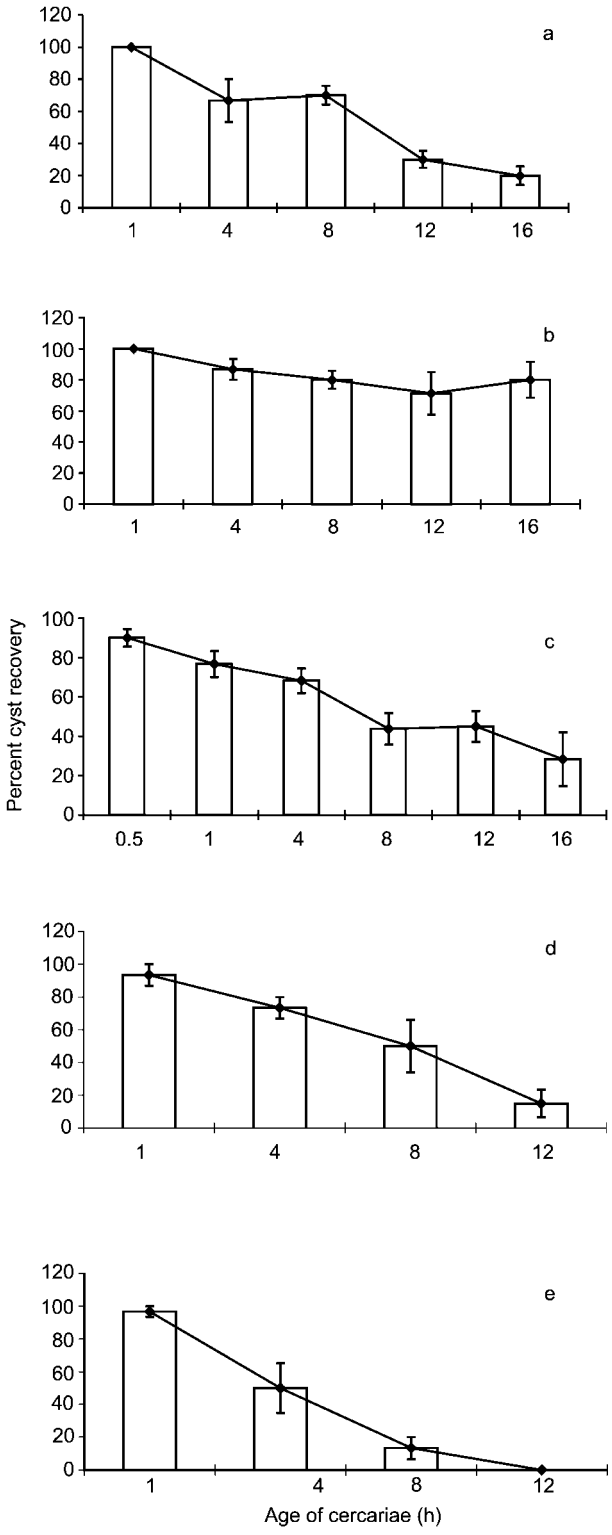

Fig. 2. a–e. Effects of temperature on infectivity (± standard error) of cercariae of *Echinostoma caproni* at different ages at (a) 4°C, (b) 12°C, (c) 23°C, (d) 28°C and (e) 37.5°C.

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As discussed by Fried & LaTerra (2002), studies on optimal temperature for *in vitro* encystment of echinostome cercariae in a defined medium are not available. The present study suggests that a temperature of 23°C is more effective than either higher or lower temperatures for the *in vitro* encystment of cercariae of *E. caproni*.

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