Journal of Helminthology (2005) **79,** 177–178 DOI: 10.1079/JOH2005278

Research Note

Cercarial tail loss in *Echinostoma caproni*: the influence of *in vivo* encystment and copper sulphate

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Abstract

Echinostoma caproni tail loss was studied *in vitro* in the presence of the toxicant copper sulphate ($CuSO_4$) in concentrations ranging from 1 to 10 000 mg l^{-1} in standardized artificial spring water (pH 7.4, osmolarity 34 mOsm kg^{-1} H_2O, Ca^{2+} 20 mg l^{-1}) at 23°C. Tail loss was also studied in the absence of toxicants during *in vivo* encystment of the cercariae in juvenile *Biomphalaria glabrata*. As the concentration of $CuSO_4$ increased, the percentage of cercarial tail loss increased. By 2 h in 10 000 mg l^{-1}, 1000 mg l^{-1} and 100 mg l^{-1} $CuSO_4$, 50%, 23% and 13%, respectively, of the cercariae had lost their tails. In the *in vivo* studies, by 1 h PI, 59 ± 5% of cercariae had lost their tails and only 4 ± 1% of the cercariae were actively swimming in the multi-well dishes. At 3 h PI, 72 ± 3% of the cercariae began to form cysts within the snails.

Tail loss in the cercariae of digeneans occurs during *in vivo* encystment in the second intermediate host and *in vitro* in the presence of toxicants. The mechanism of tail loss in the cercariae of digeneans is poorly understood. Incidental to studies on the *in vitro* effects of the toxicant copper sulphate ($CuSO_4$) on the cercariae of *Echinostoma caproni*, Reddy *et al.* (2004) noticed tail loss of cercariae, but did not quantify this phenomenon as a factor of the concentration of the toxicant. Likewise, during *in vivo* observations on encystment of *E. caproni* in juvenile *Biomphalaria glabrata* by Schneck & Fried (2004), cercarial tail loss was observed but not recorded.

Since tail loss occurs under both circumstances in *E. caproni* cercariae, i.e. *in vitro* under the influence of a toxicant and in the absence of toxicants during *in vivo* encystment in snail hosts, we decided to quantify tail loss in this study. Although studies on tail loss of cercariae have been made on furcocercous cercariae, e.g. *Schistosoma mansoni* by Hara *et al.* (1993a,b) Wiest *et al.* (1989) and *Diplostomum spathaceum* by Morley *et al.* (2002), similar studies on echinostomatid cercariae are not available. Tail loss involves a break at the tail–body junction, but mechanisms associated with this phenomenon are not well understood.

The aim of this study is to provide basic information on tail loss in the cercariae of *E. caproni* in relation to the physiology of an echinostomatid cercaria. The study was undertaken on cercariae of *E. caproni* exposed to the toxicant $CuSO_4$ under *in vitro* conditions, and during *in vivo* encystment of cercariae in juvenile *B. glabrata* in the absence of toxicants.

For the experiments to determine the effects of $CuSO_4$, all cercariae were obtained from experimentally infected *B. glabrata* (see Idris & Fried, 1996) and tested at 23°C in 24 multi-well dishes containing five cercariae per well as described in Fried & LaTerra (2002). Each well consisted of 0.5 ml of various concentrations of copper in the form of cupric sulphate pentahydrate ($CuSO_4 \cdot 5H_20$, Sigma) dissolved in artificial spring water (ASW) (Ulmer, 1970). Concentrations of $CuSO_4$ used were 10 000 mg l^{-1}, 1000 mg l^{-1}, 100 mg l^{-1} and 1 mg l^{-1}. Experiments at each concentration were replicated six times and observations were made at 0.5, 1, 2, 4, 6 and 24 h.

As the concentration of $CuSO_4$ increased, the percentage of cercariae with missing tails increased. By 2 h in 10 000 mg l^{-1}, 1000 mg l^{-1} and 100 mg l^{-1} $CuSO_4$,

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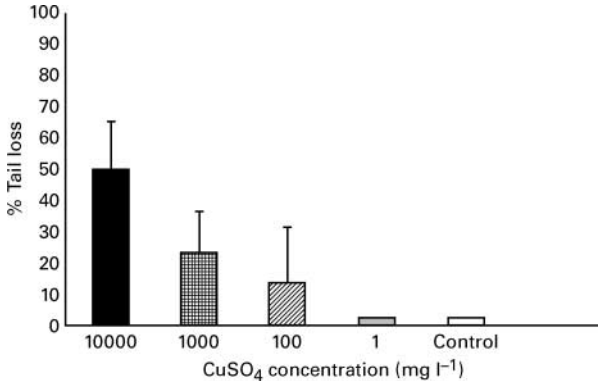

Fig. 1. Percent tail loss of *Echinostoma caproni* cercariae in various concentrations of $CuSO_4$ for 2 h. Error bars are mean ± SE; controls are in artificial spring water.

50%, 23% and 13%, respectively, of the cercariae had lost their tails (fig. 1). Cercarial tail loss in Cu concentrations of 10 000 and 1000 mg l^{-1} was significantly greater than in 1 mg l^{-1} or in the controls after 2 h of treatment (ANOVA, $P < 0.05$). Tail loss data at other time points beyond 2 h were similar and are not reported herein.

In the presence of the Cu toxicant, tail loss was induced while cercariae were still alive. Both the body and tail of newly decaudized cercariae showed intrinsic motility and responded to mechanical stimulation. This finding is in accord with that of Morley *et al.* (2002), who were able to demonstrate that tail loss in *D. spathaceum* was a distinct event occurring prior to the death of cercariae.

Further experiments were undertaken in the absence of a toxicant to determine tail loss during *in vivo* encystment. Cercariae were obtained from experimentally infected *B. glabrata* snails as described above. Four laboratory-reared *B. glabrata* juvenile snails, measuring 2–3 mm in shell diameter, were individually infected with 25 *E. caproni* cercariae in 0.5 ml ASW at 23°C in a 24 multi-well dish as described by Schneck & Fried (2004). Tail loss was assessed at 1 h post infection (PI) by counting the number of detached tails in the bottom of each well. *In vivo* encystment was monitored at 3 h PI as described in Schneck & Fried (2004).

By 1 h PI, 59 ± 5% of cercariae had lost their tails and only 4 ± 1% of cercariae were actively swimming in the multi-well dishes. At 3 h PI, 72 ± 3% of cercariae began to form cysts within the snails. Following cercarial tail loss *in vivo*, encystment of the cercarial body begins. At 3 h PI, cysts were oval in shape, lacked a cyst wall, and the larva within the cyst was moving rapidly.

The influence of various environmental parameters such as temperature and water characteristics must be taken into consideration in studies on cercarial tail loss under the influence of a toxicant (Morley *et al.*, 2002). Moreover, tail loss may be an important measurable parameter for ecotoxicological assessment (Morley *et al.*, 2003). Water quality should be as uniform as possible to obtain consistent results of the effects of the toxicant on cercarial survival and/or tail loss. In the present study at 23°C, the ASW used had a pH of 7.4, osmolarity of 34 mOsm kg^{-1} H_2O and a Ca^{2+} concentration of 20 mg l^{-1} (slightly hard water).

In conclusion, this study quantified cercarial tail loss *in vitro* as a factor of the concentration of the toxicant $CuSO_4$ and *in vivo* during cercarial encystment in experimentally infected juvenile *B. glabrata* snails in the absence of toxicants.

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(Accepted 3 January 2005)