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Influence of cadmium exposure on the incidence of first intermediate host encystment by *Echinoparyphium recurvatum* cercariae in *Lymnaea peregra*

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Abstract

The effect of cadmium exposure of the snail first intermediate host *Lymnaea peregra* on the incidence of encystment of *Echinoparyphium recurvatum* (Digenea: Echinostomatidae) cercariae without emergence from the snail was investigated. Exposure to 100 μg l^{-1} Cd for 72 h caused a significant increase in the incidence of first host encystment when compared to controls. In addition, autometallographic staining of *E. recurvatum* daughter rediae and developing cercariae showed that there was metal accumulation within their body tissues. The significance of these findings to parasite transmission in metal-polluted environments is discussed.

Introduction

Environmental conditions are major controlling forces in the life cycles of parasitic helminths. For trematodes, in particular, environmental changes can have a significant effect on transmission success (Pietrock & Marcogliese, 2003). To improve reproductive potential some trematode taxa may use the snail host as both first and second intermediate host (Poulin & Cribb, 2002), which for some species may be a conditional life history strategy, adopted only under poor environmental conditions. In echinostomes an abbreviated life cycle in which cercariae encyst without emerging from the first intermediate host has occasionally been reported (Rees, 1932; Wesenberg-Lund, 1934; Lo, 1995). Rees (1932) considered that this phenomenon was stimulated by an unfavourable environment, as cercarial encystment was related to a decrease in seasonal temperature.

Little is known about the effect of toxic chemicals on cercarial maturation and emergence into the snail host (Morley *et al.*, 2003c). Yescott & Hansen (1976) exposed *Biomphalaria glabrata* infected with *Schistosoma mansoni* to manganese and found that the sporocysts contained mature cercariae which appeared sluggish or immobile and 83% of snails contained no emerging cercariae within the haemolymph.

In the present study the *Echinoparyphium recurvatum*/*Lymnaea peregra* model was used to investigate if toxic chemicals could induce first intermediate host encystment. *Echinoparyphium recurvatum* has frequently been capable of metacercarial encystment within its first intermediate host (Evans *et al.*, 1981), and several snail host sites can be used for cercarial encystment (Adam & Lewis, 1992). However, invasion of the visceral mass occurs from within a snail already harbouring a primary *E. recurvatum* infection, as it has not been experimentally observed in uninfected snails (Adam & Lewis, 1992).

Therefore the effect of cadmium exposure to *L. peregra* on *E. recurvatum* first host encystment was investigated, including histochemical studies on daughter rediae to establish whether cadmium influences maturing cercariae or only those cercariae which emerge into the snail tissue. Cadmium is a common constituent of industrial and mining effluent. In polluted freshwater in the UK some of the highest recorded concentrations have been recorded from the River Tawe, Wales where concentrations have been as high as 160 μg l^{-1} (Vivian & Massie, 1977).

Materials and methods

A stock solution of 100 mg l^{-1} cadmium was prepared by dissolving cadmium chloride ($CdCl_2.5/2H_2O$) (Sigma Chemicals) in distilled water to give the correct concentration of metal ions. Test solutions of 100 μg l^{-1} were

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obtained by diluting the stock solution in distilled water. Synthetic soft water (25 mg l^{-1} $CaCO_3$, pH 7.85) was prepared using procedures described by HMSO (1969).

Lymnaea peregra, naturally infected with *E. recurvatum* were sampled from a site known to have a high prevalence of this parasite – Bushy Park, Greater London (National Grid Reference TQ160694). Cercariae were identified according to Nasir (1984) and Adam (1991).

Samples of 100 μg l^{-1} concentration test solutions were analysed for metal loss from soft water incubated at 20°C after 24 h. Solutions were analysed on a Perkin Elmer Optima 3300 Inductively Coupled Plasma-Atomic Emission Spectrometer which was calibrated with a 1% nitric acid blank and a standard which consisted of 1000 μg l^{-1} of cadmium in 1% nitric acid. The accuracy of the data was assessed by analysing certified reference material (NIST SRM 1643d) along with the samples and this was calculated to have a relative error of 0.00037% for cadmium.

The toxicity of cadmium to first host encystment and daughter rediae of *E. recurvatum* was investigated by screening collected snails individually in small glass vials containing soft water. Those having a mature infection of emerging *E. recurvatum* cercariae were removed from the population. The remaining snails were maintained in soft water at 20°C in an aerated aquarium and screened every 48 h. Snails that had developed an infection of emerging cercariae during this time were used for the experiment (snail size 11–15 mm).

Snails were individually placed for 72 h in 200 ml of soft water containing 100 μg l^{-1} cadmium at 20°C. Water was changed every 24 h and snails were fed on small amounts of lettuce as required. Distilled water was added as a control. Four snails were used in each exposure. After 72 h the snails were killed and dissected and the number of metacercarial cysts occurring in the visceral mass was counted. In addition, daughter rediae were removed, rinsed briefly in 0.8% saline, fixed in Bouins solution then transferred to 70% alcohol. The autometallography staining technique of Danscher *et al.* (1987) was used and modified for whole mounts and the type of silver enhancer kit (Sigma Chemicals) used in the present study, as previously described by Morley *et al.* (2003a). Whole mounts of rediae were rehydrated to distilled water, stained for 5–8 min using the silver enhancer kit, rinsed in distilled water, fixed in 2.5% sodium thiosulphate for 3–4 min, rinsed in distilled water, dehydrated, cleared in histoclear and mounted on glass slides. In addition, the staining of rediae which had been embedded in wax and sectioned was also undertaken. Cadmium was visualized as black deposits on the parasite surface. Results were square root transformed and statistically analysed using a Kruskal-Wallis one-way ANOVA with the UNISTAT software package.

Results

Chemical analysis of the test solution showed that there was only a small loss of metal ions over 24 h, which did not exceed more than 10%.

Exposure of snails to 100 μg l^{-1} cadmium for 72 h caused an increase in the number of cysts found in the visceral mass when compared to controls which, in most examples, amounted to almost three times as many cysts (control mean number of cysts: 100.0, range 64–149; 100 μg l^{-1} Cd mean number of cysts: 309.5, range 180–678). The difference in cyst number between 100 μg l^{-1} cadmium exposure and controls was significant (Kruskal-Wallis one-way ANOVA, $\chi^2 = 5.333$, $P = 0.021$).

The staining of daughter rediae (fig. 1) revealed cadmium accumulation in several sites. Considerable accumulation was apparent in the intestinal contents of each redia (fig. 1a) with 'pin-point' staining occurring around the 'rim' of the muscular anterior pharynx

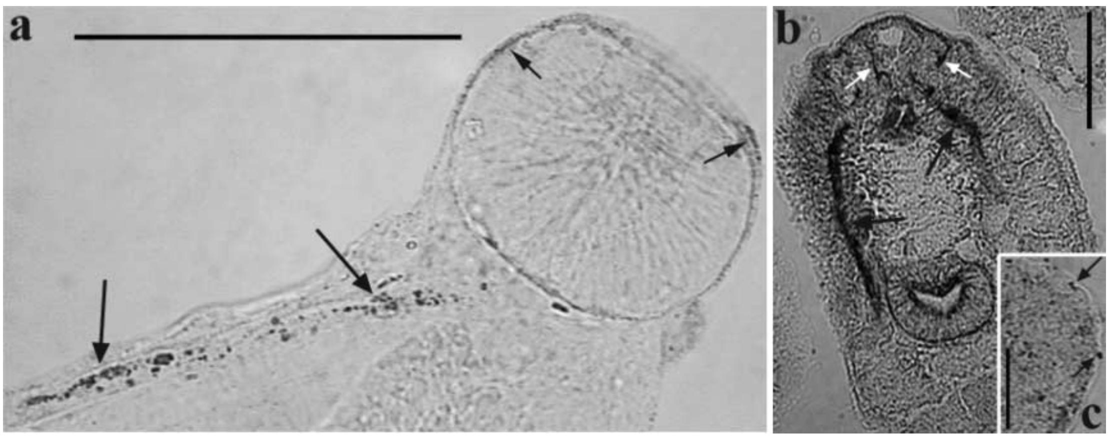

Fig. 1. Autometallography staining of cadmium in intramolluscan stages of *Echinoparyphium recurvatum*. a. Whole mount of daughter redia with extensive staining of intestinal contents (large arrows) and 'pin-point' staining around the rim of the pharynx (small arrows) (scale bar = 100 μm). b. Section of maturing cercaria in daughter redia; note staining of ventral sucker, primary excretory ducts (large black arrows), and small anterior ducts (small white arrows) (scale bar = 50 μm). c. 'Pin-point' staining (arrows) of section of anterior region of developing cercaria in the daughter redia (scale bar = 20 μm).

(fig. 1a), which may be possible sites of sensory receptors. Serial sections of maturing cercariae within the daughter redia showed staining of the ventral sucker, primary excretory ducts and small ducts in the anterior region which may be associated with either the paraoesophageal glands or excretory system (fig. 1b), and several 'pin-point' stains on the body surface (fig. 1c). Control daughter redia showed no staining on any part of their surface or on maturing cercariae.

Discussion

The present study has demonstrated that *E. recurvatum* within the snail host can be susceptible to metal exposure. Cadmium toxicity induces an increased encystment within the first intermediate host. This suggests that cercariae released into snail tissue containing cadmium are possibly reacting to the unfavourable environment by encysting before emergence into the water occurs. This response, previously noted in overwintered snails harbouring *Cercaria 'Z'* by Rees (1932), indicates that the parasite probably has a sophisticated relationship with the environment outside its snail host and will react to fluctuations in favourable conditions by modifying its release and encystment behaviour. However, Morley *et al.* (2003b) showed that in short term exposures, *E. recurvatum* demonstrates no significant change in the number and pattern of emergence from cadmium exposed *L. peregra*. Therefore probably only a minority of mature cercariae released into the snail tissue from the daughter redia may react to the unfavourable environmental conditions by encysting. This might be an evolutionary strategy to ensure that whatever the status of the freshwater environment, at least a certain proportion of the cercarial population will form metacercariae. This is despite encystment within the first intermediate host possibly leading to an overall reduced transmission success, as *L. peregra* harbouring a primary infection may only form a small percentage of the snail population and therefore *E. recurvatum* will have a reduced chance of transmission to the definitive host. Indeed, the number of cercariae that can be produced in an infection when the parasite remains in the first intermediate host is likely to be considerably less than would be produced if cercariae leave the snail (Poulin & Cribb, 2002). The consequences of changes in parasite transmission strategies in metal-polluted conditions therefore requires further study.

Autometallographic staining of daughter rediae has shown that cadmium can accumulate in several parts of the parasite. Extensive cadmium staining of the intestinal contents is not unexpected. Daughter rediae occur within, and feed on, the digestive gland of their host, which is the major organ of metal accumulation in snails (Abd Allah *et al.*, 1997). Living in a metal-saturated environment leads to some cadmium accumulation in the developing cercariae. Cross *et al.* (2001) demonstrated that cercariae from snails living in a metal-polluted site had a reduced swimming rate and survival compared to cercariae from snails in an unpolluted site. Therefore the long-term consequences of metal accumulation on parasite development within a snail host, and its effects on the subsequent transmission success of emerged cercariae, requires further study.

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