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Changes in survival characteristics of *Diplostomum spathaceum* cercariae emerged from cadmium-exposed *Lymnaea stagnalis*

N.J. Morley*, M. Crane and J.W. Lewis

School of Biological Sciences, Royal Holloway, University of London, Egham, Surrey, TW20 0EX, UK

Abstract

The effect of exposing *Lymnaea stagnalis* (Gastropoda: Pulmonata), infected with *Diplostomum spathaceum* (Trematoda: Diplostomatidae), to 100 $\mu g\,l^{-1}$ cadmium for 7 days on survival characteristics (survival, tail loss, decaudized cercarial life-span) of emerged cercariae was investigated. Exposure of *L. stagnalis* to cadmium resulted in significantly increased *D. spathaceum* cercarial survival and an inhibited tail loss compared to controls. The normal parallel relationship which exists over time between decreasing cercarial survival and increasing tail loss in controls was changed in cercariae from cadmium-exposed hosts with an increased proportion of cercarial deaths occurring without tail loss. The decaudized cercarial life-span over the survival period of the cercarial population did not significantly change. However comparisons between individuals decaudized during the initial 24 h time period with those which were decaudized during the final period of cercarial survival showed a significantly altered life span which did not occur in the control population. As a potential indicator of penetration 'fitness' comparisons were also undertaken between control and exposed cercariae decaudized during the initial 24 h time period, which revealed that the decaudized cercarial life-span from the exposed hosts was significantly different from controls. This may have important implications for the ability of cercariae to migrate through the tissues of their target host. The importance and relevance of these results to parasite transmission are discussed.

Introduction

The effect of toxic pollutants on parasite transmission and host–parasite relations is an increasing area of concern in the field of ecological parasitology (e.g. Sures, 2004). Recent studies have focused on pollutant toxicity to the transmission of larval trematodes through their snail hosts, especially those trematode species of medical or veterinary importance (Morley *et al.*, 2003d). The majority of studies have examined only the direct effect of pollutants on free-living stages and little is known about the impact of host exposure on the subsequent viability of these stages. The importance of biotic factors on parasite transmission under toxic conditions should not be underestimated (Morley & Lewis, 2004), yet there have been relatively few investigations on this subject to date. Both intramolluscan development and cercarial emergence are inhibited by toxicants (Hira & Webbe, 1972; Massoud *et al.*, 1973; Yescott & Hansen, 1976; Ibrahim *et al.*, 1992; Rondelaud, 1995; Morley *et al.*, 2003c,d,e), whilst the horizontal swimming rate and longevity of cercariae emerged from snails collected at a metal-polluted site were reduced compared to those from an unpolluted site (Cross *et al.*, 2001).

The present study therefore investigates how exposure of the snail host to cadmium may affect the survival characteristics of emerged cercariae. This forms part of a larger ecotoxicological programme on the effects of metal

*Fax: 01 784 470756
E-mail: n.morley@rhul.ac.uk

toxicity on cercariae of *Diplostomum spathaceum* (Morley *et al.*, 2001, 2002a,b, 2003a,b,c, 2005). These studies have revealed several important physiological factors that influence parasitism under toxicant exposure including the proposed use of decaudized cercariae as an indicator of 'fitness' of larvae, in terms of glycogen utilization, for migrating through the tissues of the target host (Morley *et al.*, 2003b).

Diplostomum spathaceum is an ubiquitous parasite of fish throughout the UK and occurs in many different climate zones (Chubb, 1979). Cercarial survival and tail loss are temperature dependent (Lyholt & Buchmann, 1996; Morley *et al.*, 2001, 2002a) and under most conditions are linked with a decrease in survival preceded by an increase in tail loss (Morley *et al.*, 2002a). Optimal cercarial infectivity occurs between 0 and 5 h post emergence (Whyte *et al.*, 1991).

The aims of the present study are to assess how exposure of the snail host, *Lymnaea stagnalis*, to cadmium may effect the survival characteristics of emerged cercariae, i.e. cercarial survival, tail loss, decaudized cercarial life-span. Cadmium is a common constituent of industrial and mining effluent. In the UK some of the highest recorded concentrations (160 $\mu g\,l^{-1}$ Cd) in polluted freshwater occur in the River Tawe, Wales (Vivian & Massie, 1977).

Materials and methods

A stock solution of 100 $mg\,l^{-1}$ cadmium was prepared by dissolving cadmium chloride ($CdCl_2.5/2H_2O$, minimum 98%) (Sigma Chemicals) in distilled water to give the correct concentration of metal ions. The test solution was obtained by diluting the stock solution in distilled water and adding it to the test vessels at a concentration which gave final snail exposures of 100 $\mu g\,l^{-1}$. Synthetic soft water (25 $mg\,l^{-1}$ $CaCO_3$, pH 7.85) was prepared using procedures described by HMSO (1969).

Samples of 100 $\mu g\,l^{-1}$ Cd 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 $\mu g\,l^{-1}$ of cadmium in 1% nitric acid. The accuracy of the data was assessed by analysing a certified reference material (NIST SRM 1643d) along with the samples and was calculated to have a relative error of 0.00037% for cadmium.

Lymnaea stagnalis naturally infected with *Diplostomum spathaceum* were collected from Dinton Pastures Country Park, Reading, UK (NGR SU779724). Cercariae were identified according to morphological criteria described by Niewiadomska (1986). Infected snails were allowed to acclimate to laboratory conditions in aerated aquaria containing soft water (HMSO, 1969) before being randomly split into two groups. Group one ($n = 4$) was exposed to 100 $\mu g\,l^{-1}$ cadmium in 500 ml of soft water at 20°C for 7 days. Group two ($n = 4$) acted as a control group placed under the same conditions but exposed only to distilled water. Solutions were renewed daily and snails fed on small amounts of lettuce as required.

After 7 days snails were briefly rinsed in distilled water before cercariae were collected. Cercarial survival characteristics from cadmium-exposed and control snails were examined and analysed as previously described (Morley *et al.*, 2001, 2002a, 2003b). Briefly, toxic effects were investigated by pipetting 36 cercariae (maximum age 25 min) individually into wells of flat-bottomed 96-well microtitre plates (Life Sciences International) using a wide-bore pipette tip to prevent cercarial damage. Each well contained a final solution volume of 300 μl of soft water. Cercariae were maintained in a 12 h light/dark incubator at 20°C. Three replicates of each treatment were established and observations on cercarial survival, tail loss, and decaudized cercarial survival were undertaken at intervals of 3 h. Once the cercariae had shed their tails they were observed at the same time intervals until death occurred. Death was pronounced when decaudized cercariae failed to respond to mechanical stimulation with a fine needle. Results were analysed using a Lee-Desu Comparison test for cercarial survival, tail loss and decaudized cercarial survival and for comparing changes in the tail loss/survival relationship. This test calculates survival function directly from the continuous survival times and the resulting estimates do not depend on the grouping of data into a certain number of time intervals. In addition, we investigated whether aged cercariae from cadmium-exposed snails have altered the subsequent lifespan of decaudized cercariae by comparing those individuals which decaudized during the initial 24 h survival period and the final 24 h survival period in the control and test solutions using the Lee-Desu comparison test. To investigate the relative 'fitness' of decaudized cercariae to migrate through the skin membrane of their target host, the lifespan of individuals which had decaudized only during the initial 24 h post emergence period was compared with controls using the Lee-Desu comparison test (glycogen utilization by cercariae during this period has been shown to primarily occur from the tail reserves rather than the body reserves as described by Ginetsinskaya (1960) and Fried *et al.* (1998)). In addition, a Tukey's significant difference test was used to analyse cercarial survival and tail loss for 5-h-old cercariae (the age limit of maximum cercarial infectivity). Results were analysed using UNISTAT 4.5 (Unistat Ltd, 1996).

Results

Water analysis revealed that over 24 h there was only a small loss (10%) of dissolved metals from the test solution. During the exposure period there were no mortalities of the snail hosts. Studies of the survival characteristics of cercariae from the cadmium-exposed hosts demonstrated some changes compared to controls.

Exposed-host cercariae had significantly increased survival over time when compared with controls (fig. 1) (Lee-Desu test, $P = 0.0058$), whilst exposed-host cercarial tail loss had a significantly slower rate than controls (Lee-Desu test, $P < 0.0001$) with an increased amount of inhibited tail loss, i.e. cercariae that died without shedding their tails (fig. 2a). In addition, examination of the cercarial tail loss/survival relationship over time (fig. 2b) showed that in control cercariae this was a related relationship over the lifespan of the cercarial population

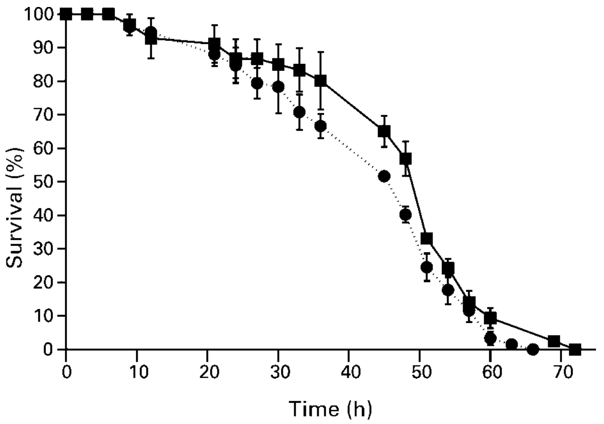


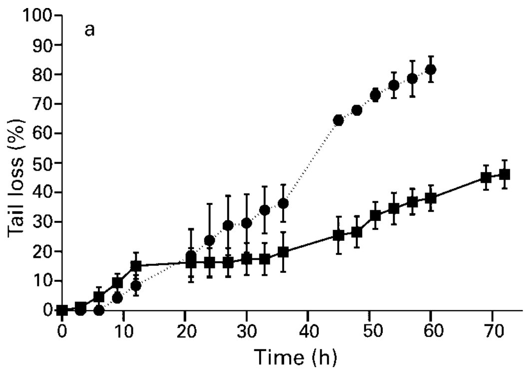
Fig. 1. Survival of *Diplostomum spathaceum* cercariae emerged from *Lymnaea stagnalis* exposed to 100 $\mu g\,l^{-1}$ cadmium for 7 days (■) or controls (●). Error bars are standard errors.

with a decrease in survival matched by a parallel increase in tail loss (Lee-Desu test, $P = 0.5049$). However, with exposed cercariae a significantly altered relationship now occurred (Lee-Desu test, $P = 0.0011$) with an increased proportion of deaths occurring without tail loss. There was no significant difference in survival or tail loss in 5-h-old cercariae between control and exposed treatments. Studies on the decaudized cercarial population revealed no overall difference in the life span between the two experimental populations (Lee-Desu test, $P = 0.3301$) (fig. 3). However, an examination of changes in the decaudized cercarial life-span as the population aged showed a significant difference in the life-span of decaudized cercariae from exposed hosts between those decaudized in the initial 24 h exposure period compared to those in the final 24 h period of survival which did not occur in the control population (Lee-Desu test, control cercariae $P = 0.4114$; exposed host cercariae $P < 0.0001$). In addition, changes in the decaudized cercarial life-span which occurred during the initial 24 h exposure period, and hence may be indicative of their penetration 'fitness', showed that there was a significantly increased life-span of exposed-host decaudized cercariae compared to controls (Lee-Desu test, $P = 0.0060$).

Discussion

The present study has demonstrated that cercariae which emerge from hosts exposed to cadmium have significantly different survival characteristics compared with controls. Morley *et al.* (2004) found accumulation of metals in the parasite intramolluscan stages and developing cercariae of *Echinoparyphium recurvatum* when the snail host *Lymnaea peregra* were exposed to cadmium. This was associated with the location of the parasite within the digestive gland of the host, which is the main organ of metal accumulation under polluted conditions (Abd Allah *et al.*, 1997). It is likely that in the present study a change in the survival characteristics of cercariae from exposed hosts is a product of metal accumulation by developing cercariae within the snail, rather than an alteration in their stored glycogen, as they are considered to retain their ability to accumulate glycogen throughout their development regardless of the detrimental conditions in the snail habitat (Chernogorenko, 1982). A similar assumption was made by Cross *et al.* (2001) studying cercariae which emerged from snails inhabiting a metal-polluted field site. In the present study, such metal accumulation whilst within the snail host has resulted in an increase in cercarial survival and inhibited tail loss. Similar findings, which were reported after direct exposure of cercariae to metals (Morley *et al.*, 2001, 2002a), have been associated with the activity of cercariae having been reduced by a probable inhibition of glycogen utilization (Morley *et al.*, 2003a). The present study suggests that metal binding to developing cercariae may cause a reduced activity on emergence from the snail host leading to an extended life span, most likely caused by the same metal inhibiting physiological mechanism associated with direct metal exposure of cercariae.

Alterations in tail loss patterns may also be attributed to metal binding to developing cercariae in the snail host inducing a reduced activity once emerged. Rea & Irwin (1992) considered that an increased tail loss in *Cryptocotyle lingua* cercariae at higher temperatures was related to increased cercarial activity, which in turn increased stress on the body–tail linkage, causing it to break. With reduced cercarial activity there is less stress on the body–tail linkage leading to fewer examples of tail loss.



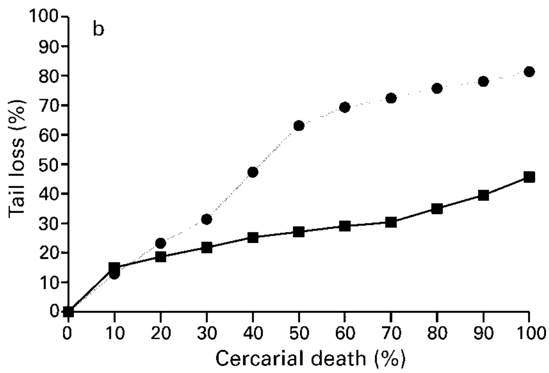


Fig. 2. Tail loss of *Diplostomum spathaceum* cercariae emerged from *Lymnaea stagnalis* exposed to 100 $\mu g\,l^{-1}$ cadmium for 7 days (■) or controls (●). (a) Tail loss over time. (b) Comparison of the relationship between cercarial tail loss and death in the experimental population. Error bars are standard errors.

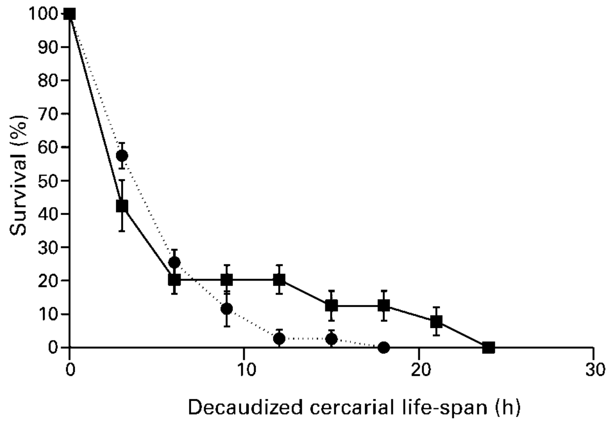


Fig. 3. The decaudized cercarial life-span of *Diplostomum spathaceum* emerged from *Lymnaea stagnalis* exposed to $100\,\mu g\,l^{-1}$ cadmium for 7 days (■) or controls (●). Error bars are standard errors.

There was a more limited effect on decaudized cercarial life-span after host exposure. This suggests that differences exist between metal exposures of cercariae within the snail and directly within a test solution, where a general trend of reduced life-span is found (Morley *et al.*, 2003b). Under direct metal exposure conditions, a reduction in the life-span of metal exposed decaudized cercariae demonstrated in previous studies (Morley *et al.*, 2003b) may be associated with increased permeability after tail loss (Howells *et al.*, 1975). This would cause cercariae to be more vulnerable to toxicants present in the water leading to a more rapid and widespread inhibition of glycogen utilization, although this did not occur in the present study. However, where changes in the life-span of decaudized cercariae did occur between control and exposed host cercariae it is still more likely to be associated with a limited inhibition of glycogen utilization. Indeed, assuming decaudized cercariae are influenced by toxic metals in the same way as the subsequent life stage (diplostomules) that penetrate and migrate through their target host tissues, it is possible that cercariae from exposed hosts would demonstrate a reduced penetration 'fitness' compared to controls. Nevertheless this assumption requires further investigation.

The changes noted in survival characteristics of cercariae from exposed snail hosts, associated with metal accumulation whilst within the snail, may therefore have long term consequences not only for transmission to the next host, but also for their eventual development into gravid adults. Such potentially far-reaching consequences need to be taken into consideration when evaluating the relative toxicity of a pollutant to any parasite life stages.

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