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Research Note

Echinostoma friedi: the effect of age of adult worms on the infectivity of miracidia

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Abstract

The effect of ageing of adults of *Echinostoma friedi* (Trematoda: Echinostomatidae) on the infectivity of miracidia yielded was analysed. Miracidia were obtained after hatching of eggs obtained from adult worms of *E. friedi* collected weekly during the course of experimental infections in golden hamsters. Miracidial infectivity, measured in terms of percentage of infection in *Lymnaea peregra*, was significantly influenced by the age of the adult worms from which the miracidia were derived. Infective miracidia only were obtained from adult worms in the age range from 4 to 9 weeks post-infection. Infectivity was maximal in those miracidia derived from adults collected 8 and 9 weeks post-infection. The results suggest that adult worms producing viable eggs require additional maturation to be able to yield eggs containing infective miracidia.

The transmission of many digeneans depends upon the ability of their miracidia to invade the snail first intermediate host. Many studies have been made to analyse the factors which determine the miracidial infectivity of several trematode species (Anderson *et al.*, 1982; Waadu, 1991; Theron *et al.*, 1998; Toledo *et al.*, 1999a; Muñoz-Antoli *et al.*, 2002). However, these studies deal mainly with factors related to the miracidial age, environmental conditions and the snail first intermediate host. No attempt has been made to analyse the miracidial infectivity in relation to factors depending on the adult worms from which these miracidia were derived. Recent studies have shown that age-dependent changes occur in *Echinostoma friedi* (Trematoda: Echinostomatidae) that affect the reproductive success of this species in the definitive host, mainly due to variations in egg output and the viability of the eggs produced (Toledo *et al.*, 2003). This raises the question of whether these changes may also affect the infectivity of the miracidia yielded. The present study was undertaken to examine the effect of adult worm ageing of *E. friedi* on the ability to produce not only viable eggs but also eggs containing infective miracidia.

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Echinostoma friedi (originally obtained from the Albufera Natural Park of Valencia, Spain) was maintained in the laboratory using *Lymnaea peregra* as the first intermediate host. This snail species was used as second intermediate host and golden hamsters (*Mesocricetus auratus*) were used as definitive hosts. Further techniques for the mainteinance of *E. friedi* in the laboratory have been described by Toledo *et al.* (2000).

The effect of adult ageing on the infectivity of the miracidia produced was investigated as follows. Each of 33 golden hamsters, weighing 45–60 g, was infected by stomach tube with 100 metacercariae of *E. friedi* collected from specimens of *L. peregra*. At each week post-inoculation (wpi) from the 1st to 12th wpi, three of the hamsters were necropsied and the worms recovered each week were pooled. The uteri of ten worms were teased to obtain eggs. Eggs were placed in Petri dishes containing 10 ml of spring water and maintained at 20 ± 1°C in the dark. At days 10–15 of incubation, egg cultures were exposed daily to artificial light (60 W) for 2 h and miracidia were obtained after the eggs hatched. Laboratory-reared specimens of *L. peregra* (size range: 0.3–0.5 cm) were singly exposed to five newly hatched miracidia (maximal age 15 min) derived from adults of *E. friedi* collected each week of the experiment. Exposures were conducted in Petri dishes for 12 h in

3 ml of spring water at 20 ± 1°C. After exposure, snails were maintained in a day:night rhythm of 12:12 h at 20 ± 1°C and were fed washed lettuce ad libitum. At 5–6 weeks post-exposure, individual snails were investigated daily to shed cercariae. Those snails that failed to shed cercariae were crushed and examined for infection. Twenty replicate exposures were conducted for miracidia derived from each adult age class. The Chi square test was used to compare the percentages of infection obtained with the miracidia yielded by each adult worm age; $P < 0.05$ was considered as significant.

All the hamsters experimentally exposed to metacercariae of *E. friedi* became infected. The number of worms recovered each week of the experiment ranged from 9 to 25 (15.7 ± 3.9) worms per hamster. All worms collected on the 1st wpi were not gravid. Eggs were obtained each week from the 2nd wpi until the end of the experiment. However, miracidial hatching was not obtained for eggs derived from adult worms collected on the 2nd, 10th and 12th wpi. The infectivity observed in miracidia derived from adults collected each week of the experiment are shown in fig. 1. The percentages of infection were not homogeneous during the course of the experiment and varied with adult worm age. Miracidia derived from adult worms collected on 3rd and 11th wpi were not infective. Considering the remaining worm age classes, the percentage of infection ranged from 20% (on 7th wpi) to 100% (on 8th wpi). Application of a χ^2 test showed that the infectivity of miracidia derived from 8-week-old adults was significantly higher than that observed in the miracidia derived from the remaining worm age classes. Moreover, statistically significant differences were observed between the infectivity of miracidia derived from 9-week-old adults and that of those obtained from adults collected on the 5th and 7th wpi.

In the present study, we evaluated the effect of *E. friedi* worm ageing on the infectivity of miracidia. The results obtained, using miracidia derived from adult worms of different ages, clearly demonstrated that miracidial success, measured in terms of percentage of infection, was significantly influenced by adult worm age. We have also shown that adult survival time overestimated the time for which worms were able to produce infective miracidia. This indicates that transmission of *E. friedi* is only likely during a limited period of its life-span as suggested by Toledo *et al.* (2003).

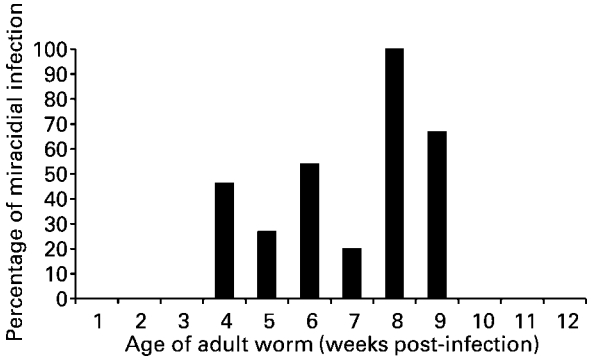

Fig. 1. Percentage infection observed with miracidia of *Echinostoma friedi* derived from eggs obtained from adult worms collected up to 12 weeks post-infection.

Toledo *et al.* (2003) demonstrated that age specific changes occur in *E. friedi* adult worms affecting the reproductive success of this species. These authors also showed that the mechanisms determining egg output and the production of viable eggs are not concomitant. The present results suggest the existence of specific mechanisms involved in the production of infective miracidia. The maximal egg output of *E. friedi* in golden hamsters occurred in the 3rd wpi with a percentage viability of about 20–30% (Toledo *et al.*, 2003). In contrast, no infective miracidia were obtained from eggs derived from adults collected that week. This suggests that adult worms producing viable eggs require further maturation to yield eggs containing infective miracidia, a situation that may be comparable to that described for several echinostome cercariae (Evans & Gordon, 1983; McCarthy, 1999; Toledo *et al.*, 1999b; Muñoz-Antoli *et al.*, 2002). These cercariae require a period of maturation after shedding to be infective. This pre-infective period has been related to shifts in gene expression (Pechenik & Fried, 1995). In the case of adult worms, a delay in reaching the ability to yield eggs containing infective miracidia may indicate that further functional changes occur after the egg release starts. Unfortunately, the extent to which these functional changes occur has not been assessed for any trematode species.

The existence of specific mechanisms involved in the production of infective miracidia is supported by the fact that the maximum yield has been observed in adult worms collected on the 8th and 9th wpi. Interestingly, this period coincides with a significant decline in the production of viable eggs in *E. friedi* (Toledo *et al.*, 2003). Although we cannot evaluate the mechanisms involved in these processes, an increase in miracidial infectivity can be viewed as advantageous since it may compensate for a reduction in viable egg production. This may help enhancement of parasite transmission in spite of worm ageing.

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