Research Note

Thermal tolerance of *Hymenolepis diminuta* eggs does not limit the parasite's distribution

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Abstract

Eggs of the tapeworm, *Hymenolepis diminuta*, and adults of the beetle, *Tenebrio molitor*, were incubated at high and low temperatures. The tapeworm's eggs survived at higher and lower temperatures and for longer periods of time than did adult beetles, indicating that the thermal tolerance of the eggs does not limit the parasite's distribution.

Most cyclophyllidean cestodes have similar life cycles. Adult worms are found in the small intestine of the definitive host, eggs are passed in the definitive host's faeces, the eggs are ingested by an intermediate host, and the life cycle is completed when the intermediate host (containing a metacestode) is eaten by the definitive host. The only stage of the parasite's life cycle that is exposed directly to the environment is the egg (or oncosphere), and if the life cycle is to be completed the egg must be more tolerant to adverse environmental conditions than the intermediate host. In this study we examined the hypothesis that the eggs of *Hymenolepis diminuta* are more tolerant than the parasite's intermediate host (*Tenebrio molitor*) to extreme temperatures, and, therefore, that the thermal tolerance of eggs is not a limiting factor in the distribution of this parasite.

The OSU strain (Pappas & Leiby, 1986) of *H. diminuta* was maintained in male Sprague-Dawley rats and grain beetles (*T. molitor*). Eggs were collected from rat faeces using NaCl flotation, washed in several changes of tap water, and stored in water at room temperature. Beetles were maintained on wheat bran supplemented every few days with small pieces of potato. Beetles were starved for at least 24 h before they were infected, and they were infected by feeding them a mixture of eggs and apple scrapings.

To determine the thermal tolerance of eggs, a suspension of eggs was added to a 15 conical centrifuge tube, the eggs were allowed to settle, and the excess water was removed; such samples routinely contained a total volume of 1 ml, consisting of settled eggs covered with a layer of water. The centrifuge tube was then placed in a circulating water bath maintained at the appropriate temperature (± 0.1°C). The temperatures of samples containing only water equilibrated with the water bath within 2 min at the highest and lowest temperatures used. Following incubation, the tube was allowed to cool or warm to room temperature, and an aliquot of the eggs removed for 'mechanical hatching'. In some instances the remaining eggs were fed to starved beetles. 'Mechanical hatching' of eggs was accomplished using a Dounce homogenizer (Pappas & Durka, 1991).

A similar protocol was used to assess the thermal tolerance of beetles. Beetles (five) were selected randomly from cultures, placed in a 50 ml round bottom centrifuge tube, and the tube sealed loosely with aluminum foil. The tube was submerged to within 20 mm of the top in a circulating water bath and incubated for an appropriate amount of time. The temperatures of tubes containing no beetles equilibrated with the water bath in 3 min or less. After incubation, the beetles were removed, allowed to equilibrate to room temperature, and examined after

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30 min. Several controls, consisting of five beetles incubated as above for 24 h at 22°C, were run. None of these beetles died.

Samples of hatched eggs (oncospheres) were examined microscopically for movement of the oncospheres. At least 500 oncospheres were examined, and samples containing moving oncospheres were considered viable; viability was not quantified. Beetles infected with eggs were examined for cysticercoids no sooner than 14 days PI. If cysticercoids were recovered from the beetles, the eggs were considered infective. Beetles that had been incubated at different temperatures were allowed to recover at room temperature for 30 min. If none of the beetles was moving at that time, they were considered dead.

For beetles to be infected with *H. diminuta*, the beetle must ingest the tapeworm's eggs, and the eggs must be broken open (the shells 'cracked') by the beetle's mouth parts. At the present time, there is no way to quantify either the numbers of eggs actually eaten or broken open by the beetles. Thus, it was impossible to quantify infectivity of the eggs (i.e. number of eggs eaten versus number of cysticercoids recovered). Moreover, the data for viability of eggs and beetles were not quantified, since we were interested in those conditions that were lethal to eggs and beetles. Thus, the eggs and beetles were classified as alive (viable) or dead, and the data were not amenable to statistical analysis.

Egg samples were fed to beetles to ensure that those samples with motile (viable) oncospheres were infective, and that those samples with no motile oncospheres were not infective. Of the 20 samples fed to beetles, 14 contained eggs that had been heated and six contained eggs that had been frozen. Without exception, beetles that were fed viable eggs were infected with cysticercoids, and beetles that were fed samples containing no motile oncospheres were not infected with cysticercoids. Thus, viability and infectivity were considered to be synonymous.

Eggs of *H. diminuta* survived at higher temperatures and for longer periods than did beetles (fig. 1). Moreover, the maximum temperatures at which eggs and beetles survived for 24 h were 39°C and 36°C, respectively. Eggs of *H. diminuta* were very resistant to low temperatures. They survived for 18 h at −15°C, but the temperature at which they would die was not determined. Beetles, on the other hand, did not survive longer than 15 min or 30 min at −15°C and −10°C, respectively.

Previous studies on the thermal tolerances of eggs of cyclophyllidean cestodes are reviewed briefly by Smyth (1969) and Smyth & McManus (1989), and 'the few reports available suggest that eggs of cyclophyllids are remarkably resistant to temperature' (Smyth, 1969). In terms of dispersal and reproductive success, it would advantageous for the eggs of a parasite to be able to survive at higher and lower temperatures and for longer periods of time than the parasite's intermediate host, and this is what is found in the case of *H. diminuta* and its intermediate host, *T. molitor*. Thus, the thermal tolerance of *H. diminuta* eggs does not limit the parasite's distribution, and this explains, in part, why this parasite 'is almost worldwide in distribution' (Burt, 1980).

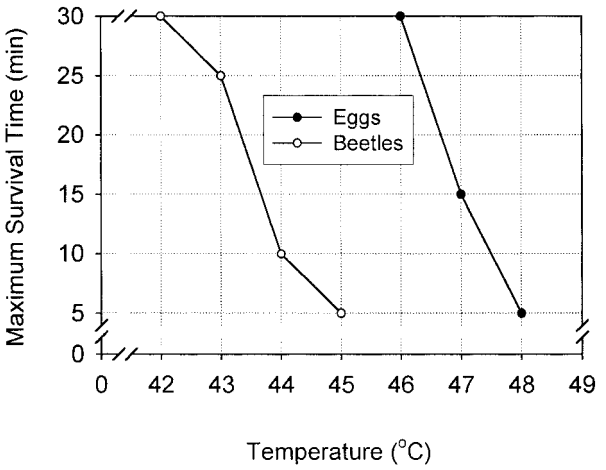

Fig. 1. Maximum time of survival of eggs of *Hymenolepis diminuta* and adult *Tenebrio molitor* at different temperatures. Samples of eggs and beetles were incubated at different temperatures for 5 min intervals, and the maximum time they survived at each temperature is indicated.

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