The influence of second intermediate host species on the infectivity of metacercarial cysts of *Echinoparyphium recurvatum*

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Abstract

The potential influence of second intermediate host species on the infectivity of metacercarial cysts of *Echinoparyphium recurvatum* to the definitive host *Anas platyrhynchos* was examined experimentally. *Echinoparyphium recurvatum* metacercarial cysts were obtained from the following experimentally infected second intermediate hosts 14 days post expsoure to cercariae: *Lymnaea peregra*; *Physa fontinalis*; *L. stagnalis; Planorbis planorbis*; *Biomphalaria glabrata*; tadpoles of the amphibian *Rana temporaria*. Metacercarial cysts from each of these hosts were fed, in doses of 50 cysts per individual, to separate groups composed of between four and eight, 3-day-old *A. platyrhynchos* ducklings. All *A. platyrhynchos* were necropsied 15 days post-infection and the number, size, and reproductive status of *E. recurvatum* worms in the intestine was recorded. Analyses of variance on the number (transformed log $(x+1)$) and size of worms revealed no significant differences in worms originating from metacercariae formed in the different second intermediate hosts (worm number $P>0.05$, and worm size $P>0.05$). All worms recovered were found to be gravid. It is therefore concluded that the species of second intermediate host utilized does not influence the infectivity of the metacercarial cyst of *E. recurvatum*, nor the subsequent establishment and reproductive status of the parasite in *A. platyrhynchos*.

Introduction

Echinoparyphium recurvatum is a 45 collar-spined echinostome digenean, the adult of which is an intestinal parasite of a wide range of aquatic birds. In Britain, aquatic birds reported as hosts include mallard (*Anas playrhynchos*), tufted duck (*Aythya fuligula*), mute swan (*Cygnus olor*), and moorhen (*Gallinula chloropus*), (see Beverley-Burton, 1972). The first intermediate hosts are known to be the aquatic pulmonate gastropods *Radix pereger* (= *R. ovata*) and *Radix auricularia* (*Lymnaea peregra* and *L. auricularia*) (see Rašin, 1933; Grabda-Kazubska & Kiseliene, 1989; McCarthy, 1989). The range of second intermediate hosts includes the tadpoles of the amphibian *Rana temporaria* and a wide range of freshwater molluscs (Rašin, 1933; Vojtková, 1963; Evans *et al*., 1981; Grabda-Kazubska & Kiseliene, 1989; Evans & Gordon, 1983; McCarthy, 1989) .

An epidemiological field study by Evans *et al*. (1981) examined the distribution and occurrence of metacercarial cysts of *E. recurvatum* in seven species of molluscs at Harting Pond, Sussex, England. These authors drew some conclusions about the relative contribution made by each species of molluscan second intermediate host towards the flow of *E. recurvatum* between the first intermediate host *L. peregra* and the aquatic bird definitive hosts at the study site. The conclusions made were subject to the assumption that the cysts contained in each host species were equally infective to definitive hosts. However, although Evans *et al*. (1981) stated that all the cysts examined during their study appeared to be normal, they also noted that the infectivity of *E. recurvatum* cysts from different species of host had not been investigated experimentally. The present study therefore set out to investigate experimentally the infectivity of *E. recurvatum*

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metacercarial cysts derived from a range of different second intermediate hosts towards the definitive host *Anas platyrhynchos*. Since experimental studies (Evans & Gordon, 1983; McCarthy, 1989) have shown considerable variation in the degree of compatibility expressed between the cercaria of *E. recurvatum* and different species of second intermediate host mollusc, it was decided to investigate the possible influence both of high compatibility second intermediate hosts (*L. peregra*, *Physa fontinalis* and *Biomphalria glabrata*) and also that of low compatibility second intermediate hosts (*L. stagnalis* and *Planorbis planorbis*) on metacercarial cyst infectivity.

Materials and methods

Laboratory bred, infection-free, specimens of the freshwater gastropods *Lymnaea peregra*, *Physa fontinalis*, *L. stagnalis*, *Planorbis planorbis* and *Biomphalaria glabrata* (Puerto Rican strain), and laboratory bred, infection-free tadpoles of *Rana temporaria*, were exposed *en masse* in species groups in synthetic hard water medium (HMSO, 1969) to cercariae of *E. recurvatum*. The cercariae were collected within 30–45 min of their emergence from naturally infected first intermediate host *L. peregra* snails collected in September 1986 from Harting Pond, West Sussex, England. Between 20 and 40 specimens of each host were exposed to infection. All snails used were in the length/diameter size class 4–7 mm. Post-exposure, the hosts were maintained at 20°C on a diet of clean boiled lettuce. At 14 days post-exposure metacercarial cysts were obtained by dissecting hosts using fine steel needles. Batches of cysts from each second intermediate origin were fed in doses of 50 cysts per bird, in gelatine capsules, to separate groups composed of either four, six or eight, 3-day-old, Khaki Campbell ducklings (*Anas platyrhynchos*). The ducklings were then maintained on a diet of non-medicated chick crumbs and water fed *ad libitum* and were sacrificed by cervical dislocation 15 days post-infection. The intestine of each bird was removed from pylorus to cloacal opening and then transferred to a surgical tray containing warm (40°C) saline (0.75% NaCl). The intestine was then divided into sections, each one of which was individually examined for parasites in a separate petri dish of fresh warm saline. Worms recovered from each intestine were counted, rinsed in clean saline, and then fixed and relaxed in Berland's fluid. The worms were then cleared, temporarily mounted in 'Ralmount' and measured using a microscope equipped with an ocular micrometer in order to provide an estimate of the projected body area (length × maximum (mid-acetabular) width) of each worm. The reproductive status of each worm (i.e. gravid or non-gravid) was recorded. Experimental infections of ducklings in this study were carried out under authorization of a Home Office licence (ELA 24/8194).

Results

The results indicate that the species of second intermediate host utilized by *E. recurvatum* has no significant influence on the subsequent infection success of the parasite in the wildfowl definitive host *A. platyrhynchos* (table 1). Analyses of variance on the number of worms recovered from each host 15 days post-infection (transformed $\log(x+1)$), and the estimated body areas of worms, revealed no significant differences between the worms derived from the six different second intermediate host species. The results of the analyses of variance were respectively: worm number $P > 0.05$; worm size $P > 0.05$. All the worms recovered in this study, irrespective of second intermediate host origin, were found to be gravid indicating that second intermediate host origin is likely to have little influence on the eventual reproductive status of the parasite in the definitive host.

Discussion

The results of this study suggest that for the range of hosts examined, the species of second intermediate host utilized by *E. recurvatum* is unlikely to have any significant effect on the infectivity (per metacercarial cyst ingested) of the parasite to the definitive host *A. platyrhynchos*. Experimental studies of this nature are rare and therefore the scope for comparison of results is limited. However, Christensen *et al*. (1980) carried out a similar study on the echinostome *Echinostoma caproni* (referred to as *Echinostoma liei* in their paper). These authors compared under experimental conditions the infectivity of metacercarial cysts from 14 different second intermediate host snail species to laboratory mice. Using as a criterion of infectivity the mean number of worms recovered per host expressed as a percentage of the initial

Table 1. The influence of second intermediate host species on the infectivity of *Echinoparyphium recurvatum* metacercarial cysts to *Anas platyrhynchos*.

Second intermediate host origin of metacercarial cysts	No. of ducklings exposed to infection*	Mean no (± standard error) worms recovered per duckling 15 days post-infection**	Mean no. (± standard error) estimated area per worm (mm^2)
Lymnaea peregra	6	11.3 (± 1.2)	2.4 (± 0.1)
Physa fontinalis	6	10.3 (± 1.2)	2.3 (± 0.1)
Planorbis planorbis	4	11.0 (± 2.9)	2.3 (± 0.1)
Lymnaea stagnalis	4	9.5 (± 2.5)	2.1 (± 0.2)
Biomphalaria glabrata	6	12.3 (± 3.1)	2.3 (± 0.1)
Rana temporaria (tadpole)	8	8.5 (± 3.2)	2.1 (± 0.2)

*50 cysts per duckling.
** All worms were gravid at necropsy.

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cyst infection dose, Christensen *et al*. (1980) arrived at the same conclusion for *E. caproni* that is made for *E. recurvatum* in the present study; metacercarial infectivity to the definitive host is independent of the species of second intermediate host utilized.

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