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Effects of a 100 metacercarial cyst inoculum on the host–parasite relationship of *Echinostoma caproni* and ICR mice

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Abstract

The host–parasite relationship of a 100 metacercarial cyst inoculum of *Echinostoma caproni* in the ICR mouse was examined. Three groups of mice, A, B and C, each with six mice per group were used and all mice were necropsied at 14 days postinfection (p.i.), at which time the worms were ovigerous. Group A consisted of uninfected controls, whereas group B received 25 cysts per mouse (low dose) and group C received 100 cysts per mouse (high dose). There was no significant difference in food consumption between any of the groups from 0 to 14 days p.i. Control mice increased their body weight by 12%, group B by 5%, and group C showed a less than 1% increase in body weight between 0 and 14 days p.i. Echinostome parasitism caused a significant increase in the diameter of the mouse gut, with the gut of group C being more significantly dilated than that of either group A or B. The average worm recovery from group B was 20 worms per host, compared to 72 worms per host from group C. The mean wet and dry weights per worm from group B were 2.4 and 0.4 mg, respectively as compared to 0.6 and 0.2 mg respectively for group C. The mean number of uterine eggs per worm from group B was 180 compared to 125 for worms from group C. Worms from group C were more widely distributed in the small intestine than those from group B. Crowding effects associated with the high dose infection were clearly demonstrated in *E. caproni* from ICR mice.

Introduction

Numerous studies in our laboratory have used the Institute of Cancer Research (ICR) mouse exposed to 25 metacercarial cysts of *Echinostoma caproni* with recoveries of 15 to 20 worms per host at 2–3 weeks postinfection (see review in Fried & Huffman, 1996). This level of infection has minimal overt effects on ICR mice, i.e. hosts do not suffer from cachexia or anorexia, and show weight gains similar to the uninfected controls; qualitatively, they also consume the same amount of food as the uninfected controls.

Although Manger & Fried (1993) studied the effects of a 100 cyst inoculum (considered a high dose) on preovigerous worms of *E. caproni* in ICR mice, similar work with ovigerous echinostomes of this species is not available. In the study reported herein, we examined the host–parasite relationship of the high dose on ICR mice infected for 2 weeks at which time this species is ovigerous. Specifically, we examined the effects of such an inoculum on food consumption, weight gain and the intestinal diameter of ICR mice. We also examined the effects of this inoculum on worm growth, uterine egg production and distribution of these echinostomes in the host gut.

Materials and methods

Encysted metacercariae of *E. caproni* were maintained in laboratory infected *Biomphalaria glabrata* snails and

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used within 2 weeks post-infection (p.i.). Cysts were dissected from the kidney/pericardium region of the snails. Female ICR mice, 8 weeks old were used as hosts. In the experiment, six mice were maintained as uninfected controls (group A), six were fed by stomach tube each with 25 metacercarial cysts (group B), and six were fed the same way with 100 cysts (group C). All mice were caged individually, given water *ad libitum* and fed a measured weight of standard rodent food (Prolab Animal Diet, PMI Feeds Inc., St Louis, Missouri, USA) containing 23% protein, 32% carbohydrates, 11% fat and minimum daily requirements of vitamins and minerals. We determined the weight in grams of food consumed for each mouse throughout the infection period. Each mouse was provided with 30 g of food for a 3 day period beginning at day 0, i.e. the day they were fed cysts until day 14 p.i. at which time the mice were necropsied. Weights of the mice were determined in grams during the 14 day period. Mice were weighed individually on day 0, and then on days 7 and 14 p.i. At necropsy, on day 14 p.i., the small intestine was removed from the pylorus to the ileocaecal valve, and the diameter of the intestine was measured in centimetres at the widest zone. The intestine was opened longitudinally and separated into five equal sections beginning at the pylorus. Worms were counted from each section and placed in Petri dishes containing Locke's solution. For growth studies, some worms were fixed without flattening in hot (80–90°C) alcohol-formalin-acetic acid (AFA), stained in Gower's carmine, dehydrated in ethanol, cleared in xylene, and mounted in Permount. Body and organ measurements (in mm) were made with a calibrated ocular micrometer on each of ten randomly selected worms from group B and C. For uterine egg counts, some worms were placed on a slide, flattened with moderate coverslip pressure and the slide was placed in a Petri dish containing AFA for 2 h; these worms were prepared as whole mounts as described above. Egg counts were made on each of nine randomly selected flattened worms from group B and C. The remaining worms were used for wet and dry weight measurements; worms were blotted on a paper towel to remove excess water and separated into five groups, each with ten worms per group and the weights were determined on an analytical balance. This procedure was done for worms from group B and C. Once the wet weights were determined, the worm groups were placed in a 42°C incubator for 24 h and dry weights were taken. Final weight determinations were based on wet and dry weights in mg per worm. Student's *t*-test ($P < 0.05$ being considered significant) was used to compare differences in weights, food consumption and intestinal diameter in control versus experimental mice. The *t*-test was also used to compare worm weight and body measurements of echinostomes from groups B and C.

Results

In preliminary experiments, six ICR mice were each exposed to (not reported in detail herein) 175 ± 25 cysts. Three showed signs of cachexia and anorexia and died on day 10 p.i., prior to necropsy. The other three also showed signs of anorexia and cachexia, but survived until necropsy on day 14 p.i. These mice contained from 90 to 158 worms per host. Because of our experience with cyst inocula greater than 100 per host, we decided to use an inoculum of 100 cysts per host as the high cyst dose in the experiments reported herein.

Table 1. Mean ± SE in grams of food consumed per mouse at 3 day intervals of control and experimental ICR mice.

	Groups		
No. of observations*	A	B	C
1	10.2±2.0	10.2±1.1	10.6±0.9
2	13.2±1.1	13.5±0.7	12.8±0.7
3	16.7±0.8	17.6±1.4	16.2±1.0
4	10.0±1.1	9.9±1.5	9.4±1.7
5	14.9±1.0	16.3±1.2	15.5±1.3

* Each observation was made at 3 day intervals beginning at day 0 and ending on day 14 postinfection.
Group A, six uninfected controls; group B, six mice each exposed to 25 cysts of *Echinostoma caproni*; group C, six mice each exposed to 100 cysts.

There was no significant difference in the average amount of food consumed per mouse at 3 day intervals for the uninfected mice versus those that received either 25 or 100 cysts (table 1). The control mice and those that received 25 cysts showed progressive weight gain, whereas the heavily infected mice maintained their weight (fig. 1). From day 0 to day 14 p.i., the uninfected mice showed about a 12% increase in body weight; mice infected with 25 cysts showed an approximate 6% increase and mice infected with 100 cysts showed an increase of <1%. The mean ± SE diameter of the intestine of group C was significantly greater than that of group A and B. The mean ± SE of the diameter of the small intestine of group C was 2.3 ± 0.1 cm. Group A mice had a mean ± SE intestinal diameter of 1.1 ± 0.2 cm. The mean ± SE of the intestine of group B was 1.6 ± 0.1 cm.

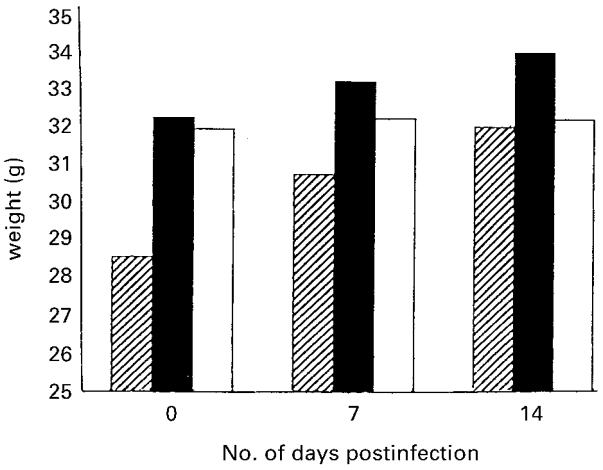

Fig. 1. Mean weight in grams of control and experimental mice at 0, 7, and 14 days postinfection with *Echinostoma caproni* (▨, control; ■, 25 cysts; □, 100 cysts).

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Table 2. Mean percentage of worms recovered at 14 days postinfection with *Echinostoma caproni* from each of five equal segments of the small intestine*.

	Segment no.				
No. of cysts	1	2	3	4	5
25	0	18	44	38	0
100	0	24	46	28	2

* n = 6 for hosts exposed to both 25 and 100 cysts; segment 1 begins at the pylorus and segment 5 ends at the ileocaecal valve.

The average worm burden was 20 worms per mouse for group B and 72 worms per mouse from group C. In group B, 82% of all worms were in segments 3 and 4, whereas 71% of all worms were in segments 3 and 4 in group C (table 2). The diameter of the acetabulum, oral sucker, width of the ovaries, and anterior testis of the worms from group B were significantly greater than that of similar measurements of worms from group C (table 3). The number of uterine eggs per worm was significantly lower in echinostomes removed from group C. The mean ± SE number of uterine eggs was 125.2 ± 3.0 from worms from group C, whereas the mean number of such eggs was 180.4 ± 2.0 for worms from group B. The wet and dry weights of worms from group B were significantly greater than those from group C. The mean ± SE wet and dry weights for worms from group B was 2.4 ± 0.01 mg and 0.4 ± 0.1 mg, respectively. The mean ± SE wet and dry worm weights from group C were 0.6 ± 0.02 mg and 0.2 ± 0.01 mg, respectively.

Discussion

Mice infected with the high cyst dose consumed food at the same rate as the control and low cyst group, yet they failed to gain weight during the two week period of infection. Probably severe gross and histopathological effects of the gut associated with the high dose caused a malabsorbtion syndrome accounting for a failure to gain weight in spite of normal food consumption. Perhaps the larger number of echinostomes in the gut resulting from the high dose caused a greater utilization of host ingesta by these worms than that seen in infections with the low cyst dose.

Table 3. Mean ± SE (mm) body and organ measurements of adult worms of *Echinostoma caproni* at 14 days postinfection.

	No. of cysts	
Measurements in mm	25	100
Body length	4.5±1.0	3.9±0.10
Body width	1.0±0.10	0.8±0.01
Body area[a]	4.5±0.10	3.1±0.10
Oral sucker diameter	2.0±0.10*	0.1±0.01
Acetabulum diameter	0.5±0.01*	0.4±0.01
Ovary length	0.2±0.01	0.2±0.03
Ovary width	0.4±0.01*	0.2±0.01
Anterior testis length	0.3±0.02	0.3±0.02
Anterior testis width	0.5±0.01*	0.4±0.01
Posterior testis length	0.3±0.01	0.3±0.02
Posterior testis width	0.5±0.01*	0.3±0.01

* Measurements were significantly greater ($P < 0.05$) in the 25 cyst group than the 100 cyst group; n = 10.
[a] Body area reported in mm^2.

Mice with heavy infections showed greater dilation of the gut than those with light infections. Some dilation of the gut has been noted in ICR mice infected with single worms of *E. caproni* (B. Fried, unpublished observations). Such dilation may result from oedema, and gross and histopathological changes associated with tissue hypertrophy and cellular hyperplasia in the gut. However, the mechanism of gut dilation in the *E. caproni*– ICR mouse model is poorly understood and needs further study.

Studies on the crowding effect of *E. caproni* in mice have not been documented, but some information on this phenomenon in *E. trivolvis* (referred to as *E. revolutum* in the paper) has been discussed by Fried & Freeborne (1984). The crowding effect has not been studied as intensely with trematodes as it has been with cestodes and acanthocephalans. Yao *et al.* (1991) found that *E. caproni* adults in golden hamsters fed a large number of cysts, i.e. 200 cysts per hamster, were stunted compared with worms obtained from hosts only fed 15 cysts per host. Stunted worms showed signs of worm crowding and reached maturity at about one half the size of the non-stunted cohorts. Yao *et al.* (1991) also noted that infection with 50 and 100 cysts had an effect on worm distribution in the hamster intestine. With heavy infections the worms become widely dispersed along the intestine. Similar findings were noted in ICR mice that received the high cyst dose in that crowding effects caused stunted worms, decrease in the number of uterine eggs and greater dispersion of the worms in the small intestine.

Because it may be advantagous to raise 50–70 sexually mature worms of *E. caproni* per host within 2 weeks p.i., our study provides information that such worm infections can be obtained with minimal overt changes in ICR mice. In contrast to this finding, our preliminary studies showed that cyst inocula of 175 ± 25 in ICR mice produced infections of 90–158 worms per host, but these mice showed obvious signs of cachexia and anorexia within 10 days p.i.

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