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Echinostoma caproni infection in non-pregnant female BALB/c and Swiss T.O. mice: effect on feed intake, liveweight, and serum pregnancy-associated murine protein-1 and corticosterone

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Introduction

Trematodes of the genus *Echinostoma* are cosmopolitan flukes living in the intestines and bile ducts of numerous vertebrates, including mammals and aquatic and semi-aquatic birds (*Huffman & Fried* 1990). The intestinal species, *E. caproni,* is particularly well adapted to the laboratory mouse which acts as an ideal model for studying the effects of intestinal trematode infection on the final host (*Hosier & Fried* 1986). *E. caproni* has been shown to impair reproductive efficiency in the mouse by adversely affecting early post-implantation pregnancy. Infection normally results in a loss of embryos between five and nine days after conception and is accompanied by a drop in circulating progesterone levels (*Bindseil & Hau* 1991). A significant reduction in serum pregnancy-associated murine protein-1 (PAMP-1), (*Hau et al.* 1981), has also been demonstrated in BALB/c mice harbouring both *E. caproni* and *Schistosoma mansoni* (*Bindseil et al.* 1991). The pathogenic effects of *E. caproni* have also been studied in a number of other strains, including NMRI (*Odaibo et al.* 1988), SS and ICR (*Christensen et al.* 1985, 1986), athymic nude mice (*Bindseil & Christensen* 1984) and ICR (*Hosier & Fried* 1986), although none of the latter was used to investigate the effect of the fluke burden on reproductive efficiency. The aims of the present study were to compare two strains of mice and the response in these strains to experimental infection with *E. caproni* with respect to (a) parasite establishment; (b) feed intake and liveweight gain; and (c) serum PAMP-1 and corticosterone concentrations in non-pregnant female animals.

Materials and methods

Animals, husbandry and feeding

Thirty, conventional inbred virgin female BALB/c mice (Charles River, UK) and 30, outbred virgin female Swiss T.O. mice (King's College, University of London) were used for the study. The animals were kept in groups of ten, each group being housed in a 41 × 25 × 12 cm macrolon cage. Both BALB/c and Swiss T.O. mice were delivered at seven weeks of age and allowed to acclimatise for three weeks prior to the beginning of the experiment (day 0) when each weighed (mean ± SEM) 19.6 ± 0.2 g and 23.0 ± 0.3 g, respectively. Within each strain, the same biomass (± 0.5 g) was assigned to each cage. All cages were placed on the top shelf of racks supported 126 cm above the room floor to minimise the effect that differences in illumination might have on activity and subsequent feed intake. Lighting consisted of a 12h:12h, light:dark cycle (06.00–18.00 h). Room temperature was maintained between 19° and 22° C and relative humidity between 33 % and 59 %. Mice cages were cleaned twice weekly. All mice were given *ad lib*-access to a non-crumbling pelleted diet R&M 3 containing 0.25 % ivermectin (SDS Ltd., Witham, Essex, UK) and tap water. Ivermectin was included in the pelleted ration to control a low level pinworm infection detected in the Swiss T.O. mice during routine screening

upon delivery. Ivermectin is not active against trematodes and not considered likely to interfere with the experimental fluke infection. Lignocel soft wood shavings (grade 3/4; RSA Services & Research Ltd., Finedon, UK) were used as bedding.

Measurement of feed intake

Pelleted feed was supplied in hoppers and the group daily intake measured by calculating the difference between the weight of feed offered with that remaining the following morning (11.00 h). Any pellet fragments found on the cage floor were included in the weighed quota.

Experimental design

On experiment day 0, 20 of the 30 mice of each strain (ie. two of the three groups) were infected with 25 metacercariae per mouse in 0.3 ml tap water by gavage. The remaining ten animals of each strain (control group) were given 0.3 ml tap water only, as a placebo. On day 39, ten of the infected mice of each strain were treated orally with 0.3 ml of a 1:1 water:praziquantel (50 mg/kg; Droncit®, Bayer) mixture (infected-treated group). Although lower doses (5–10 mg/kg) of praziquantel are effective against intestinal cestode infections, higher doses (50 mg/kg) are required to remove trematode burdens (*Andrews et al.* 1983). The remaining infected animals were given the same volume of aqueous polyethyleneglycol (PEG) solution (vehicle) as a placebo (infected only group). On day 46, all the mice were weighed and subsequently anesthetised by i.p. injection of pentabarbitone sodium (60 mg/kg). The mice were exsanguinated, serum reserved for subsequent analysis and the intestines removed and transferred to large petri dishes for further examination. The number of flukes and their location in the intestines were recorded.

Blood analyses

Serum PAMP-1 concentrations were determined by rocket immunoelectrophoresis as described by *Hau et al.* (1978). A pool of serum from parasite-free, non-pregnant female mice served as the master standard and was assigned a value of 100 arbitrary units (AU) per ml. The sensitivity of the assay was 0.05 AU/ml and the intra- and inter-assay coefficient of variation < 5 % and < 8 %, respectively. Serum concentrations of corticosterone (4-pregnen-11, 21-diol-3, 20-dione), the major glucocorticoid in mice, were measured using a commercial radioimmunoassay kit (gamma-B125 Corticosterone RIA, Biogenesis Ltd, Bournemouth, UK).

Statistical analyses

Between group differences were compared using a one-way analysis of variance (ANOVA) or, where appropriate Fisher's exact test and Tukey-Kramer Multiple Comparisons Test (GraphPad™ Instat 1.15, GraphPad Software, San Diego, USA); p-values < 0.05 were considered significant.

Results

Clinical observations

No clinical signs were associated with the fluke burden and there was no change in faecal consistency. In the Swiss T.O. groups the infected animals consumed significantly more food than the infected-treated and control animals. In the BALB/c mice the infected

Table 1. Mean mouse body weight (g) ± SEM, n = 10 per group.

	BALB/c			Swiss T.O.		
	Control	Infected	Infected + treated	Control	Infected	Infected + control
Day 0	19.2 ± 0.2	19.6 ± 0.2	19.6 ± 0.2	23.0 ± 0.3	23.0 ± 0.3	23.0 ± 0.3
Day 46	22.5 ± 0.4	21.6 ± 0.4	21.3 ± 0.4	27.3 ± 0.5	29.5 ± 0.5	28.1 ± 0.5

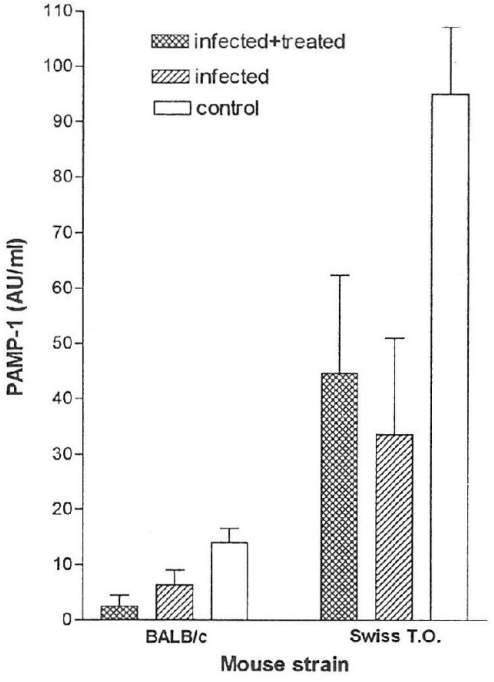

Figure 1. Serum levels of PAMP-1 on Day 46 of the experiment.

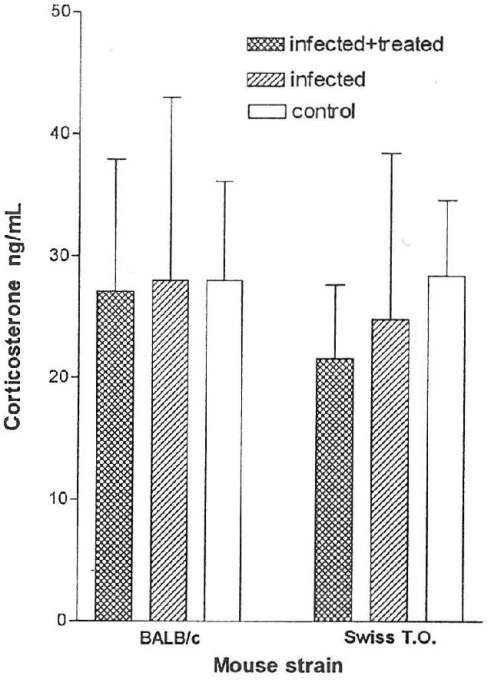

Figure 2. Serum levels of corticosterone on Day 46 of the experiment.

and the infected-treated animals consumed significantly more food than the control mice. The liveweight gain in the BALB/c mice was significantly higher in the controls than in the two other groups (Table 2). In the Swiss T.O. mice the infected mice had a significantly higher weight gain than the controls and the infected-treated animals (Table 1).

Parasitological observations

At post mortem, 4.6 ± 2.9 (mean ± SEM) and 5.5 ± 3.1 flukes were removed from the jejunum and ileum of infected BALB/c and Swiss T.O. mice representing a recovery of 19.6 % and 22.0 %, respectively. Praziquantel treatment reduced the parasite burden by 85.4 % and 100 % to 0.67 ± 0.55 (2.4 % recovery) and 0 flukes (0 % recovery) in the BALB/c and Swiss T.O. mice, respectively.

Blood analyses

Echinostoma infection resulted in a 65.0 % reduction in PAMP-1 concentrations in Swiss T.O. ($P < 0.01$) and a 56.6 % reduction in BALB/c ($P < 0.05$) mice on day 46 in comparison with control group values (Figure 1). A significant drop was also recorded in the infected-treated groups of both Swiss T.O. (46.9 % reduction in comparison with control values; $P < 0.05$) and BALB/c (81.1 % reduction; $P < 0.001$) on the same day. PAMP-1 levels in control Swiss T.O. mice were significantly higher than corresponding BALB/c mouse values ($P < 0.001$) on day 46 although there was no significant difference in concentrations between infected only and infected-treated groups within each mouse strain. Neither was there a significant correlation between fluke burden and circulating PAMP-1 values. No significant dif-

ferences were recorded in serum corticosterone concentrations between Swiss T.O. or BALB/c mice or between control, infected only and infected-treated groups (overall mean ± SEM, 33.8 ± 6.3 ng/ml) on day 46 (Figure 2).

Discussion

Although large differences in infectivity have been reported for *Echinostoma* in Swiss Webster and ICR mice (*Hosier & Fried* 1986), the recovery of *E. caproni* in the present study was relatively low compared with that reported by some workers (*Sirag et al.* 1980, *Christensen et al.* 1981). This may have been due either to low parasite infectivity, low innate susceptibility of the final host or to an earlier than expected expulsion of the parasite burden prior to post mortem examination. The inclusion of ivermectin in the diet to control pinworm infection in the Swiss T.O. mice is unlikely to have influenced fluke establishment since the drug does not appear to exhibit any activity against trematodes (*Bruce* 1987).

The relatively low fluke burdens established in mice in the present study significantly affected feed intake. In both strains of mice the infected animals consumed more food than the controls and in the Swiss T.O. mice the infected-treated animals also consumed more food than the controls. All animals gained weight during the study but in the BALB/c group the controls gained more weight than the infected and infected-treated animals in spite of the infected animals having a higher food intake. This indicates that some of the energy in the food was not utilized to growth but to meet the increased physiological strain of the infection. In the Swiss T.O. mice the higher food intake in the infected animals resulted in a larger increase in body weight compared to the normal controls.

The parasite burden depressed PAMP-1 levels in infected mice of both strains by the end of the study. Although not overall significant, praziquantel treatment seemed partly to restore circulating PAMP-1 concentrations in most infected Swiss T.O. mice and in two of the ten BALB/c animals. Clinically healthy BALB/c mice and other inbred strains have quite low PAMP-1 levels compared with outbred animals such as NMRI mice (*Hau et al.* 1985). The fact that parasite-free control BALB/c mice had significantly lower PAMP-1 values than Swiss T.O. animals was therefore not unexpected and may partly explain why blood levels in the BALB/c mice were still below the limit of detection (0.05 AU/ml) after anthelmintic treatment. PAMP-1 levels below the sensitivity of the present assay have previously only been reported in healthy adult male and juvenile females (three to 14-day-old) (*Hau et al.* 1978) and female mice harbouring both *Schistosoma mansoni* and *E. caproni* during the first eight days of gestation (*Bindseil et al.* 1991). Worm-free pregnant and non-pregnant female mice normally have relatively high blood values (*Hau et al.* 1982).

The precise mechanisms for this fluke-associated drop in PAMP-1 concentrations have yet to be identified. Blood levels of PAMP-1 are known to be regulated by growth hormone (GH) acting on the hepatic production of the protein (*Chemnitz et al.* 1982, *Hau et al.* 1990) though whether the reduction in blood values in the present study was due to an impairment in the synthesis/release of GH from the pituitary is unknown. In rats decreases in blood GH have been associated with chronic stress and accompanied by elevated circulating corticosterone levels (*Armanio et al.* 1984) though the latter were unaffected by the parasite burden in the present study (*Abraham et al.* 1994). The present study demonstrates that *E. caproni* adversely affects the production of PAMP-1, in non-pregnant female mice, suggesting that early pregnancy failure may result from parasite-induced changes that occur prior to fertilisation. The overall positive liveweight gain in the present study implies that anorexia and/or impaired digestion are unlikely to play a role in early pregnancy failure associated with *Echinostoma caproni* infection.

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Summary
The effect of experimental infection with the intestinal trematode, *Echinostoma caproni*, on feed intake, liveweight and serum pregnancy-associated murine protein-1 (PAMP-1) and corticosterone was investigated in non-pregnant female BALB/c and Swiss T.O. mice. Twenty-five metacercariae were given by oral gavage on day 0 of which 19.6 % were recovered at post mortem on day 46 in the BALB/c mice and 22.0 % in the Swiss T. O. mice. Oral praziquantel treatment (50 mg/kg) on day 39 reduced the recovery of flukes from previously-infected mice by 85.4 % and 100 % in BALB/c and Swiss T.O. mice, respectively. The food consumption in the infected Swiss T.O. mice was significantly increased compared to the infected-treated and the controls. In the BALB/c mice the food intake in the infected and infected-treated animals was significantly increased compared with the controls. The liveweight gain during the experiment in the BALB/c control mice was significantly higher than in the infected-treated groups. In the Swiss T.O. mice the infected animals gained more weight than the controls and the infected-treated animals. *E. caproni* was associated with a significant reduction in PAMP-1 concentrations in both strains of mice, even in animals from which the majority of flukes had been removed by praziquantel-treatment. There was no significant correlation between the magnitude of the parasite burden and serum PAMP-1 values. Corticosterone values were unaffected by either mouse strain, *E. caproni* infection or anthelmintic treatment. These results suggest that the previously reported adverse effect of *E. caproni* on early pregnancy in mice may be caused by parasite-induced effects occurring prior to fertilisation.

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