Effects of the tapeworm *Hymenolepis diminuta* on maternal investment in rats

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Abstract: Parasites can influence the optimal allocation of energy between present and future reproduction by changing the value of current offspring relative to that of future ones. We examined the effect of the tapeworm parasite *Hymenolepis diminuta* on maternal investment in rats. Litter sizes and average pup body mass did not differ between parasitised and nonparasitised mothers, either at birth or at weaning. The two groups of mothers also did not differ with respect to pup growth or pup mortality during lactation. Mass change during lactation was similar between parasitised and nonparasitised mothers. However, a pup-retrieval experiment showed that parasitised mothers were quicker at retrieving their pups to safety than nonparasitised mothers, and spent less time checking for potential sources of danger. These differences were particularly pronounced toward the end of the lactation period. It would appear that the parasite increases the relative value of the current litter and current levels of maternal investment, possibly because it reduces future reproductive success.

Résumé : Les parasites peuvent affecter l'allocation optimale de l'énergie entre la reproduction du moment et la reproduction dans l'avenir en changeant la valeur de la progéniture actuelle relativement à celle des progénitures futures. Nous avons examiné les effets de la présence du cestode *Hymenolepis diminuta* sur l'investissement maternel chez des rats. Le nombre de petits par portée et la masse moyenne des ratons étaient semblables chez les mères parasitées et les mères non parasitées, aussi bien à la naissance qu'au moment du sevrage. Chez les deux groupes de mères, la croissance des ratons et leur mortalité au cours de l'allaitement étaient semblables également. Les changements de masse au cours de l'allaitement étaient semblables aussi chez les mères parasitées et les mères non parasitées. Cependant, une expérience de retrait des petits a démontré que les mères parasitées étaient plus rapides à récupérer leurs petits que les mères non parasitées et passaient moins de temps à guetter les sources potentielles de danger. Ces différences étaient particulièrement marquées vers la fin de la période d'allaitement. Il semble que la présence du parasite augmente la valeur relative de la progéniture du moment et l'importance de l'investissement maternel, peut-être parce qu'elle diminue les chances de succès de la reproduction dans l'avenir.

[Traduit par la Rédaction]

Introduction

Parental investment has been defined as the care that parents invest in individual offspring which increases the survival rate of these offspring at the cost of investment in other offspring (Trivers 1972). Energy investment in the current reproductive effort are made at the expense of investment in future offspring (Williams 1966). Thus, too much or too little parental investment in current offspring can reduce lifetime reproductive success (Konig and Markl 1987). Selection has favoured patterns of optimal parental investment that are flexible, and that may be influenced by a variety of factors (Clutton-Brock and Godfray 1991; Korpimaki et al. 1994). Parasites are potentially one such factor (Forbes 1993), but their effects on parental investment have hardly been investigated, especially in mammals.

The little evidence available for mammals suggests that postnatal investment in offspring may be influenced by parasites. For instance, Weatherly (1971) studied the effects of the nematode *Trichinella spiralis* on gestation, litter size, and litter survival in mice. Results from his study show that nematode infections in mothers did not affect litter size at birth but reduced progeny survival, even at low levels of infection. Proximate reasons for these results were not given, but they are consistent with reduced postnatal investment by infected mothers. Berdoy et al. (1995) also found no difference in litter size at birth between nonparasitised female rats and females parasitised with the protozoan *Toxoplasma gondii*, an otherwise debilitating parasite. Investment following birth may therefore be more sensitive to the effects of parasitic infection than prenatal investments. Parasites should not always cause a reduction in current parental investment, however. Forbes (1993) pointed out that animals should decrease their current investment if infected by a parasite that greatly reduces the amount of resources currently available for host reproduction, but increase their current investment if the parasite is likely to decrease their life-span or their future reproduction.

Here we investigate the effects of the tapeworm *Hymenolepis diminuta* on parental investment in female rats. Adult worms live in the small intestine of rats, where they compete with the host for nutrients. Parasitised rats can suffer from a reduction in growth (Mettrick 1973), although the effects of the parasite on the host's energy budget in the wild are likely to be most acute in reproductive hosts or in hosts subjected to

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food shortage (Munger and Karasov 1989). In addition, *H. diminuta* triggers an immune reaction in parasitised rats (Hindsbo et al. 1982), and mounting this response may be costly for the host (Zuk 1996). The amount of energy required by female rats during gestation is 10–30% greater than for nonpregnant mature rats; during lactation these energy requirements become 2–4 times higher (Baker et al. 1979). The tapeworm may thus intensify the trade-off between parental condition and future reproduction on the one hand and offspring survival and current reproduction on the other. According to Forbes' (1993) predictions, because it drains currently available energy away from female rats, *H. diminuta* should tilt the balance in favour of reduced parental investment into current offspring and enhanced survivorship of the mother. We examined maternal investment from three perspectives: (1) survival and mass gain of pups; (2) relative mass change of the mother; and (3) the mother's efficiency at retrieving and protecting her offspring. This last measure may not be costly in terms of energy but may involve risks such as exposure to predators, and risk-taking parents are often also the ones investing the most energy in feeding their offspring (Rytkonen et al. 1995).

Materials and methods

Animal care and infection

All holding conditions and experimental procedures were approved by the University of Otago's Committee on Ethics in the Care and Use of Laboratory Animals. Male and female Sprague–Dawley rats aged between 4 and 5 weeks were obtained from the Department of Laboratory Animal Sciences, University of Otago. Rats were housed in individual rat breeding cages (RB3 rat cages, North Kent Plastic Cages Ltd., Kent, England; overall size 45 × 28 × 20 cm). Sawdust or wood shavings were used for bedding. Cages were cleaned twice a week. The rats were on a 10 h light : 14 h dark photoperiod; the average room temperature was 23 ± 3°C (mean ± SD). Water and food pellets were provided ad libitum.

A population of *H. diminuta* is maintained at the University of Otago by cyclical passage through Sprague–Dawley rats and the flour beetle *Tribolium confusum*. Before they reached 6 weeks of age, we infected a proportion of the female rats (11 out of 25, with the rest serving as uninfected controls) with eight cysticercoids (the larval stage of *H. diminuta*). Rats were anaesthetised with ether and cysticercoids (contained in Hanks' balanced salt solution) were introduced by stomach intubation. This procedure always results in successful infection, with typically more than half of the eight cysticercoids administered becoming established and growing into adult worms within 3–5 weeks (Pike and Chappell 1981; Willis 1998). This infection procedure has no side effects on rats, as shown in other experiments where "sham" infections were used (Willis 1998).

Experimental procedures

All animals used in the experiments were older than 11 weeks. The entire experiments were conducted in the home cage. Parasitised ($n = 11$) and nonparasitised ($n = 14$) females were each paired with randomly chosen nonparasitised males. Once pregnancy was ascertained, males were removed from the females' home cages. Pups and their mother were weighed 1 complete day after birth. This reduced infanticide due to disturbance while ensuring that mass was as close as possible to that at birth. We then weighed the pups at 7, 14, and 21 days after birth, i.e., approximately until weaning (about day 21). The mother was also weighed again on the day of weaning.

On days 5, 10, and 15 after birth, an experiment was performed to quantify maternal investment in the form of risk-taking in order to retrieve pups from an exposed area. The home cage was wheeled across the room and placed under a video camera. Each mother was given at least 15 min to settle with the pups in hiding (i.e., underneath the food and water bottle holders, usually with the pups feeding); in rare cases we terminated the experiment after 1 h because settling had not occurred. Once the female had settled, a video recorder connected to the camera was turned on and the top of the cage was then lifted, rotated 180°, and replaced on the cage so that the pups and mother were exposed. We measured the time taken by a female to retrieve all her pups to "safety," i.e., to the covered area under the food holder. During this time most females spent a portion of the time with their nose through the bars of the cage. We labelled this behaviour "checking." A long checking time means that although a female takes a long time to carry her pups to safety, the female herself is not under cover. It may represent time spent inspecting for danger. We subtracted time spent checking from retrieval time to obtain two independent measures.

Statistical analysis

We used the statistical package STATVIEW (Abacus Concepts, Berkeley, Calif.) for all t tests and correlations. We used unpaired t tests for comparisons between two groups except when data were not normally distributed. In such cases, we used two sample randomisation tests, based on 20 000 permutations computed with the RT 2.0 statistical package (Bryan Manly, Centre for the Application of Statistics and Mathematics, University of Otago). Randomisation tests are more powerful alternatives to nonparametric tests when data deviate from the assumptions of parametric tests (for details and examples see Manly 1991).

Results

Across all females, litter sizes at birth varied from 6 to 19 pups and litter sizes at weaning from 6 to 17 pups. Litter sex ratios were almost exactly 1:1 for both parasitised and nonparasitised mothers. Across all females, litter size at birth did not correlate significantly with average pup mass ($n = 25$, $r = -0.206$, $p = 0.323$). The mass of the mother 1 day after giving birth did not correlate significantly with either litter size ($r = 0.252$, $p = 0.249$) or average pup mass ($r = 0.054$, $p = 0.818$).

Parasitised and nonparasitised mothers did not differ, at birth or afterwards, with respect to their mass, their litter size, or the average mass of their pups (unpaired t tests or randomisation tests; all $p > 0.40$; Table 1). The relative mass gain during lactation did not differ between parasitised and nonparasitised mothers (randomisation test, $p = 0.147$). They also did not differ in the relative rate of pup loss ($n = 22$, $t = 0.231$, $p = 0.819$) or the average relative mass gain per pup ($n = 22$, $t = 0.529$, $p = 0.603$) during the lactation period. Parasitised mothers with a litter of 12 or more pups lost an average of 19% of their pups, whereas nonparasitised mothers with a litter of 14 or more pups lost an average of 15% of their offspring. Mothers with litters smaller than this did not lose any offspring.

In the retrieval experiments, nonparasitised mothers consistently took longer to retrieve their pups to safety than parasitised mothers (Fig. 1). However, though the difference was highly significant on day 15 after birth, it only ap-

Table 1. Characteristics of parasitised (n = 11) and nonparasitised (n = 14) rat mothers and their litters at birth and at weaning.

Variable	At birth		At weaning	
	Parasitised	Nonparasitised	Parasitised	Nonparasitised
Mother's mass (g)	313.6 (10.8)	308.2 (25.5)	342.1 (10.1)	324.9 (19.7)
Litter size	12.6 (3.8)	12.7 (1.9)	12.1 (3.5)	11.8 (1.5)
Average pup mass (g)	7.2 (0.1)	7.1 (0.1)	42.9 (4.6)	43.1 (6.4)

Note: Values are given as the mean with SD in parentheses.

Fig. 1. Time (mean ± SE) required to retrieve all pups to safety (A) and average time required per pup (B) for nonparasitised rat mothers (n = 14) and rat mothers parasitised by the tapeworm *Hymenolepis diminuta* (n = 11) as a function of time since birth of the pups. Retrieval time was computed as the time taken to retrieve the pups minus the time spent checking. Statistical comparisons were made using randomisation tests (*, $p < 0.10$; **, $p < 0.01$).

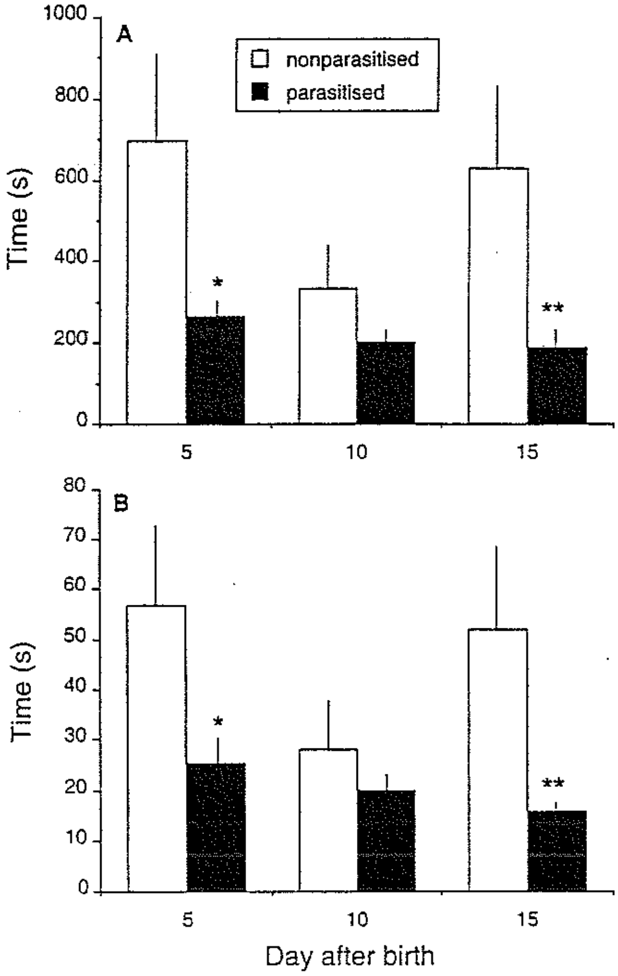
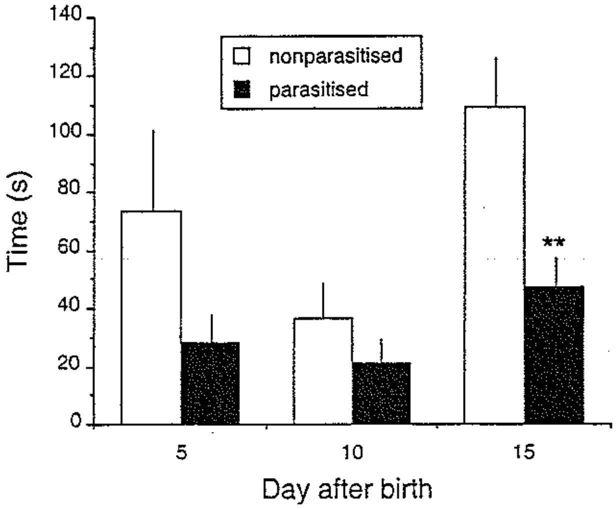


Fig. 2. Time (mean ± SE) spent checking through the bars of the cage during retrieval of pups to safety by nonparasitised rat mothers (n = 14) and rat mothers parasitised by *H. diminuta* (n = 11) as a function of time since birth of the pups. Statistical comparisons were made using randomisation tests (**, $p < 0.01$).



proached significance on day 5. Parasitised mothers also tended to spend less time checking through the bars of the cage while retrieving their pups than nonparasitised mothers (Fig. 2). There was no relationship between litter size and retrieval time among either parasitised or nonparasitised mothers (both $p > 0.15$).

Discussion

Parasites compete with hosts for energy and should therefore influence the optimal allocation of energy made by hosts to different functions, such as investment in present and future reproduction. Our results show that *H. diminuta* has no measurable effect on the number or mass of pups produced by female rats, or on their growth and survival during lactation. However, we found that parasitised mothers tended to retrieve their pups to the safety of cover more quickly than nonparasitised mothers, and to spend less time checking for potential danger. These findings suggest that parasitism influences the optimal levels of parental care provided by female rats in a way that presumably increases the lifetime reproductive success of parasitised females.

The similarity in number and size of pups produced by parasitised and nonparasitised mothers has several possible explanations. First, gestation may have taken marginally longer in parasitised females, allowing their offspring to reach the same size as those born to nonparasitised mothers. However, we detected no differences in gestation period between the two groups of females. Second, the effects of *H. diminuta* may have been masked by the ad libitum diet provided to rats (Coop and Holmes 1996). We did not quantify the food eaten by individual females, and ethical constraints prevented us from looking at the combined effects of parasitism and partial food deprivation. The unrestricted diet may also account for the similar growth rates of pups and mothers, whether parasitised or not, during lactation. It may also be that the cost of the immune response mounted by the rat against the parasite is greater when food is scarce, especially during gestation and (or) lactation. Effects of parasitism on pup quality may thus occur but be neutralised by high food intake. We did not look for more subtle effects on pup quality, such as the altered behaviour sometimes reported in offspring of parasitised mothers (Rau 1985; Sorci et al. 1994).

Differences in pup mortality rates before weaning are also potentially masked by the mother's diet. Infanticide was the main cause of mortality in the first week following birth. After the first week, pup bodies were found with no obvious marks; death probably resulted from inadequate feeding. Parasitised mothers started losing pups at a smaller litter size than nonparasitised mothers, but we had too few large litters to examine whether this trend resulted from parasitism.

Our most intriguing finding is that parasitised females were consistently quicker than nonparasitised females when retrieving their pups to the safety of cover, and spent less time checking through the bars of the cage, possibly for sources of danger. This may indicate that parasitised females are willing to put themselves at risk to increase the survival of their offspring. The effect was not statistically significant 10 days after birth; however, the trend was the same as at other times and this may simply be due to sampling error. It is interesting to note that on day 15 after birth, when differences were most pronounced, pups were more mobile than before and often could reach cover on their own. Thus, even when the efforts necessary to retrieve the pups are minimal, nonparasitised mothers take much longer to do so than parasitised ones. Long retrieval times often resulted from a mother taking much more time to retrieve the last pup than earlier ones (see also Lucion and de Almeida 1996). It may be that once most of the pups are safe, the extra benefits of saving the last pup do not outweigh the potential risk of predation on the mother. Parasitised mothers may have more to gain from saving that last pup than nonparasitised mothers, and thus they do so quickly.

All this suggests that the future reproductive success of parasitised females may be less certain than that of nonparasitised females, and that their current litter is relatively more valuable. Rats become prolific breeders from about the age of 10 weeks, and can live for about 3 years. All females used in our experiments were first-time breeders. It would seem that these females had practically their entire reproductive life ahead of them. Why should parasitised mothers take more risks to protect their first litter when they may be able to produce many more in the future? We assumed that the main pathological effect of *H. diminuta* was a reduction in the amount of energy available for the current production of offspring. Such a parasite should cause a reduction in current reproductive effort in parallel with increased investment in survival and future reproduction (Forbes 1993). Our results suggest that the opposite pattern occurs. Perhaps *H. diminuta* has longer term effects on the health of its rat host that may negatively influence the rat's future prospects, thus increasing the relative value of its current litter.

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