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Research

Infection before pregnancy affects immunity and response to social challenge in the next generation

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Natural selection should favour parents that are able to adjust their offspring's life-history strategy and resource allocation in response to changing environmental and social conditions. Pathogens impose particularly strong and variable selective pressure on host life histories, and parental genes will benefit if offspring are appropriately primed to meet the immunological challenges ahead. Here, we investigated transgenerational immune priming by examining reproductive resource allocation by female mice in response to direct infection with *Babesia microti* prior to pregnancy. Female mice previously infected with *B. microti* gained more weight over pregnancy, and spent more time nursing their offspring. These offspring generated an accelerated response to *B. microti* as adults, clearing the infection sooner and losing less weight as a result of infection. They also showed an altered hormonal response to novel social environments, decreasing instead of increasing testosterone production upon social housing. These results suggest that a dominance-resistance trade-off can be mediated by cues from the previous generation. We suggest that strategic maternal investment in response to an infection leads to increased disease resistance in the following generation. Offspring from previously infected mothers downregulate investment in acquisition of social dominance, which in natural systems would reduce access to mating opportunities. In doing so, however, they avoid the reduced disease resistance associated with increased testosterone and dominance. The benefits of accelerated clearance of infection and reduced weight loss during infection may outweigh costs associated with reduced social dominance in an environment where the risk of disease is high.

Keywords: dominance; hormones; immunocompetence; maternal effects; testosterone; trade-off

1. INTRODUCTION

Maternal effects occur when factors in the maternal environment cause phenotypic changes in offspring [1]. These effects are the phenotypic product of the interaction between the parental genotype and that genotype's environment as expressed in the next generation [2–4]. They can be viewed as manifestations of transgenerational phenotypic plasticity [5–8]. Where this plasticity increases fitness, maternal effects can be considered adaptive; adaptive maternal effects are thought to play an important role in shaping life history in many taxa [1].

The idea that the maternal environment can influence the development and hence the phenotype of offspring has attracted particular attention in studies of the impact of environmental challenges on human health. 'Foetal programming' as a result of maternal exposure to disease or malnutrition, for example, has been shown to have diverse consequences for the health of children and even grandchildren [9]. Such maternal effects in humans are typically thought of as being detrimental to offspring fitness, but in studies of other species there has been more focus on potentially adaptive priming of offspring [2,10]. If cues in the maternal environment predict the conditions likely to be experienced by the next generation, a mother's inclusive fitness can be increased if she can manipulate offspring phenotype appropriately. In many cases, it is hypothesized that maternal effects mediate life-history trade-offs in offspring involving investment in traits associated with reproductive success and survival [1]. However, the adaptive nature of maternal effects is still being actively debated. Some suggest that effects which are perceived to be adaptive may in fact simply be accidental products of gene–environment interactions with little impact on fitness [10–12].

The ability to resist or tolerate infection is crucial for animals exposed to pathogens, but it is also costly, and requires investment of resources that might otherwise be directed elsewhere [13]. Because immature animals do not have fully developed sensory and immune systems, parents often play a key role in the protection of offspring from infection. In species with parental care, protection can take several forms. Parents may directly reduce risk from pathogens through preening, nest-cleaning, etc. [14,15], and/or they may provide passive immunity by transferring antibodies to offspring in milk or via the yolk or placenta [16]. In addition, and of particular relevance here, parents may confer long-lasting protection by manipulating offspring phenotype according to the perceived risk of infection.

Transgenerational immune priming (TGIP) is a pathogen-mediated maternal effect that is known to occur in a wide range of taxa [9,17,18]. Maternal exposure to pathogens or antigens (e.g. bacterial polysaccharides) promotes offspring immunocompetence in species ranging from insects to birds and mammals [19–21]. The mechanisms that bring about TGIP are varied [9]. Some instances appear to involve optimization of maternal reproductive behaviour to benefit offspring (e.g. skewing laying order relative to offspring gender if one sex is more vulnerable to disease) [22]. Others involve direct transfer of antibodies or immunoglobulins through the milk or yolk, which can have long-term impacts on the development of the juvenile immune system, in addition to providing passive immunity [16,23]. A third group of mechanisms involves epigenetic effects, whereby maternal experience affects DNA expression, but not sequences, in offspring [24]. This may be achieved by the modulation of DNA methylation via changes in the exposure of offspring to androgens and other hormones [19,25,26], or through other biochemical mechanisms [27].

The potential for TGIP is of great interest in an evolutionary context because pathogens impose strong selective pressures on host life histories, forcing hosts to allocate resources to immune defence to the detriment of other life-history components [13,28,29]. For example, immunologically challenged female blue tits (*Cyanistes caeruleus*) and pied flycatchers (*Ficedula hypoleuca*) reduce the rate at which they feed offspring [30,31]. Other examples illustrate an opposite effect, i.e. increased investment in reproduction or growth can cause a reduction in immunocompetence. Female odour causes male mice to invest in secondary sexual characters, leading them subsequently to lose more weight fighting an experimental infection [32]. Individuals of many bird species that have larger broods show reduced immunocompetence [1]. Reproductive activity also reduces immunocompetence in fruit flies (*Drosophila melanogaster*) and damselflies (*Matrona basilaris*) [33,34].

The widespread evidence for a trade-off between reproductive traits and immunocompetence leads to clear predictions about the consequences of TGIP for offspring. If females can use environmental cues to anticipate pathogenic challenges for their offspring, and upregulate offspring immunocompetence as a result, we would expect to see the costs of this priming to impact negatively on traits associated with

reproductive success [35]. In many species, trade-offs between survival and reproduction are manifest in impacts of changes in immunity on social and reproductive behaviour. There is a well-documented negative association between social dominance and disease resistance in mice [36–38]. Aggressive interactions among male mice determine social dominance, and hence access to mating opportunities, but increased aggression is costly and is associated with reduced resistance to disease. The trade-off between investment in social dominance and immunocompetence is thought to be regulated by androgens: increased testosterone production generally increases aggression, but also increases vulnerability to infection [39]. Where such a trade-off exists, we can predict that upregulation of offspring immunocompetence as a result of TGIP by mothers may lead to decreased circulating levels of testosterone and consequently reduced social dominance.

Although pathogens have been shown to affect reproductive investment by mothers, and subsequently the behaviour and life history of offspring, such changes do not necessarily represent adaptive maternal effects. In fact, there are three possible evolutionary explanations for pathogen-mediated changes in offspring: (i) they reflect adaptive manipulation beneficial to the pathogen [40–42], (ii) they are the inevitable side-effects of the cost of combating disease [43,44], or (iii) they are adaptive responses to infection by the host ensuring their own reproductive success and that of their offspring [1]. An example of a transgenerational effect that might benefit a parasite is seen in flea-infested great-tits (*Parus major*), which produce fledglings that disperse shorter distances than healthy birds, and are more likely to be recruited to the local population, providing more nearby future hosts for the parasite [45]. If this is adaptive manipulation of host offspring behaviour by the parasite, perhaps achieved via epigenetic modifications of host DNA [24], the phenotype of the parasite can be considered to extend (*sensu* Dawkins [46]) across two host generations. In contrast, however, mite-infected female lizards (*Lacerta vivipara*) display a parasite-mediated maternal effect that appears to be adaptive for the host. They produce offspring that develop faster, invest more in early reproduction and run faster: literally and figuratively living faster and dying younger in an environment when life-expectancy is reduced [47,48]. Such examples highlight the diverse nature of the possible evolutionary origins of transgenerational effects of pathogens. However, experimental studies that trace the impacts of infection across generations such that the likely consequences of observed changes for maternal and offspring fitness can be evaluated fully remain very scarce [1].

The cause of any responses to maternal infection observed in offspring is often attributed to whichever agent (host or pathogen) is intuitively thought to benefit. Because previous experimental studies of TGIP have usually examined offspring of females that were infected at the time of pregnancy/egg laying, responses that are adaptive for the pathogen are generally very plausible, since the pathogen is still an active agent at the moment the offspring are primed [10,49]. An alternative experimental approach is provided by tests of the downstream effects of TGIP in the offspring

of females that have cleared infection prior to pregnancy/egg laying; such an approach allows us to focus more on changes in offspring traits that might plausibly be adaptive for the mother.

The environmental cues that trigger TGIP might be direct if the mother is infected prior to the birth of her offspring, but they might also be indirect. An important possible source of indirect information about infection risk in the environment comes from visual or olfactory cues associated with infected conspecifics [50]. In a previous experiment [51], we examined whether such indirect cues, when perceived by mothers, actually impact on offspring phenotype. We showed that housing pregnant female mice next to infected neighbours led to the production of offspring that were more resistant to disease and less aggressive as adults. This provided the first evidence for transgenerational regulation of immune response based on ambient social information. In addition, our findings supported the idea that mothers adaptively regulate the trade-off between social dominance and disease resistance in their offspring in response to variable infection risk.

In the current paper, we report on a second study designed to investigate transgenerational transmission of immunological information using the same experimental system as before. This time we examined the response in mothers and their offspring to direct evidence of infection risk in the maternal environment. We infected female mice with the protozoan blood parasite *Babesia microti*, and allowed the infection to clear prior to pregnancy. Thus, females in the experiment had direct information about infectious agents in the environment, and potentially had reduced resources owing to recent infection, but any priming of their offspring could only occur after infection had cleared, in the absence of the parasite. We examined how recent infection affects reproductive output and maternal behaviour, and how it affects the social behaviour and disease resistance of offspring as adults in later life. We predicted that we would see enhanced immunocompetence but reduced testosterone, aggression and social dominance in offspring of infected mothers.

2. MATERIAL AND METHODS

(a) *Methods overview*

Female laboratory mice were either infected with the blood protozoan *B. microti*, or subjected to one of four control treatments. *Babesia* species are tick-borne haemoprotozoan parasites that infect virtually all mammalian species, with significant economic consequences in domestic animals and human health implications, particularly in the USA [52,53]. In mice, *B. microti* induces high but transient parasitaemias, which are quickly cleared (clearance beginning approximately 10 days after infection) [54]. This makes *B. microti* an ideal parasitic tool to investigate the effects of disease in neighbours over a limited period, such as during pregnancy. In natural infections, natural killer (NK) cells and macrophages act to limit the extent of parasitaemia [55] before a resolution stage begins, with parasitaemia levels peaking and then rapidly declining owing to the action of CD4$^+$ T cells and IFN-γ [54]. Following primary infection, mice are protected against future infection by the

action of CD4$^+$ T cells and IFN-γ, with little or no requirement for B-cells or antibodies [56].

In our experiment, following clearance of infection (time to clearance was less than or equal to 14 days (see [51])), each female was co-housed with a sire male for 6 days. Mated females were then removed to single-housing cages until parturition. At 11 days old, each litter was reduced to four males. Females were removed from offspring at 24 days old. At 50 days old, approximately half of the offspring were re-housed with three novel males from the same treatment group. Continuous behavioural observations totalling 200 min per group were carried out, recording aggressive behaviour in the adult offspring. At 70 days old, the adult offspring were singly housed and infected with *B. microti*. The time course of the infection was monitored by blood smears until clearance. Blood was taken from females and offspring at various time points during the experiment and assayed for testosterone, corticosterone and total IgG.

(b) *Detailed methods*

(i) *Mice and housing*

A priori power tests were conducted to determine minimum sample sizes necessary to detect treatment effects of a magnitude that would be considered important, in order to minimize animal use. The subjects were 358 mice of the Bantam and Kingman: White (BKW) strain, which included 75 subject females (seven weeks old), 75 sire males (nine weeks old) and 208 male offspring from the subject females (from birth to 90 days of age). We kept subjects in standard polypropylene cages ($48 \times 15 \times 13$ cm: model M3, North Kent Plastics, UK) throughout. All cages contained wood shavings as a floor substrate, a cotton nestlet for bedding material and a cardboard tube. Subjects had ad libitum access to standard laboratory rodent food pellets and water. Room temperature was maintained between 20°C and 22°C and humidity between 45 and 55 per cent. All animals were maintained under a 12 L : 12 D reversed light : dark cycle with lights on at 20.00 h, and illuminated by a dim red light during the dark cycle to facilitate observations.

(ii) *Maternal treatment phase*

Seven week old females were randomly divided into five treatment groups of 15 individuals each. These were: (i) *Babesia* treatment: infection with 5×10^7 mouse red blood cells harbouring *B. microti*; (ii) sham *Babesia*: sham infection of *B. microti*, comprising only the vehicle used to suspend erythrocytes in treatment (i) (an infection vehicle control treatment); (iii) sheep red blood cell (SRBC) treatment: inoculation with 5×10^7 foreign SBRCs (an immune activity treatment); (iv) sham SRBC treatment: sham inoculation of SRBCs, comprising only the vehicle used to suspend the SRBCs (immune activity vehicle control treatment); and (v) complete control: no treatment. The treatment phase lasted 20 days, by which time the infection had peaked and been cleared in all of the infected females (*Babesia* treatment) (the infection monitoring method is explained in §2*b*(vii)).

(iii) *Mating phase*

The females were then introduced to the home cages of randomly allocated single-housed sire males, and kept in these pairs for 6 days. This method was used to minimize sire–female aggression, and maximize likelihood of impregnation [57]. After 6 days, females were removed from sire cages and housed singly to continue pregnancy and then give birth. They were then kept with full litters for 11 days.

(iv) *Pre-weaning phase*

At 11 days old, when pups could be reliably sexed by non-invasive means and individually marked, all were sexed and each litter reduced to four males (three males in the case of three litters that had only three males each). This was to enable manageable sample sizes for individual observations both at pre-weaning and in subsequent phases. Male pups were chosen because previous work has shown important dominance–resistance trade-offs in male mice [36–38]. A total of 208 male pups from 52 females were included in the adult offspring stages of the experiment. Thirteen females did not become pregnant in the mating phase, and a further 10 females did not have at least three sons at 11 days post-partum, but neither the likelihood of becoming pregnant nor litter sex ratio were affected by treatment (see §3). We marked the male pups in an individually distinctive pattern using black eyelash dye (Colorsport, Brodie and Stone plc, London, UK). At 24 days of age, all mothers were removed from litters, and pups (subject males) were left in their fraternal groups until 50 days old.

(v) *Social grouping and single-housing phases*

At 50 days old, all subject males were separated from their siblings. Approximately half of the subject males (100 mice) were randomly chosen and re-housed with three novel males from the same treatment group, with whom they were allowed to establish dominance hierarchies over 20 days. Continuous behavioural observations totalling 200 min (10 min d^{-1}) per group were carried out. Various social interactions were recorded, including the number of attacks, mounts and aggressive allogrooms, in order to determine social rank within these groups. Individuals that displayed more aggressive behaviours were considered dominant in later analysis, and those that were more commonly the recipients of these behaviours were considered subordinates [36,58]. The remaining half (108 mice) was housed singly to act as a non-socialized treatment. At the end of this phase, all males were housed singly in new clean cages.

(vi) *Offspring infection phase*

At 70 days old, the adult offspring, all now housed singly, were injected with 1×10^7 mouse red blood cells infected with *B. microti*. The time course of the infection was closely monitored until clearance. No other infection or immune challenge was given to the adult offspring.

(vii) *Technical procedures*

All inoculations, infections and sham manipulations involved a single intra-peritoneal injection of 200 μl Hanks' solution, containing the appropriate inoculants. Mothers in treatment group E received no injection. All monitoring, sampling and handling for treatments A and C were repeated accordingly in sham treatments B, D and E. Sham infection and inoculation involved the introduction of Hanks' solution only. All females and subject male offspring were infected in a random order with 1×10^7 mouse red blood cells infected with *B. microti*. The King's 67 strain of *B. microti* was used throughout, and frozen stock was passaged five times in BKW mice before being used on stimulus or subject males.

To monitor *B. microti* infection, a peripheral tail vein was nicked and a single drop of blood transferred to a glass microscope slide every other day during the infection. The drop was immediately smeared to give a monolayer of erythrocytes, then fixed and stained. For staining, fixed slides were placed in a solution of one part Giemsa stain to three parts Sorenson's buffer for 40 min, before rinsing in Sorenson's buffer and drying in air. Larger blood samples (50 μl) were also collected in heparinized haematocrit tubes, on no more than three occasions per animal, and never twice within a two week period. Females were sampled at the start and at the end of the stimulus phase. Offspring had 50 μl blood samples taken at weaning, prior to and at the end of social grouping. An additional blood sample was taken from all animals during autopsy.

The larger blood samples were assayed for testosterone (males only), corticosterone and total IgG (used as a bystander measure of immunocompetence [36]) using kits or reagents supplied by IDS Ltd, Tyne and Wear, UK (testosterone); R & D Systems Europe Ltd, Abington, UK (corticosterone); and Universal Biological, Cambridge, UK (IgG). All plates were processed using Micro plate Manager v. 5.2. In all cases, blood samples were anonymized and analysed in a random order, so that it was not possible for investigators to know the treatment group or relatedness of individual samples. In a number of cases, limited serum volumes precluded reliable estimates for all three serum factors at all time points from certain individuals. As a consequence, sample sizes of some analyses vary.

(viii) *Statistical analysis*

Analysis was carried out using SPSS v. 15 (SPSS Inc. Chicago, IL, USA). Alpha was set at 0.05 and all tests were two-tailed. Where appropriate, values were nested within female (which was included as a random factor), or averaged per female to avoid within-litter pseudoreplication. Where appropriate, body mass was fitted as a covariate. In addition to sample size variation owing to blood sampling limitations, sample sizes vary among early phase analyses (for example, changes in female physiology over infection phase, compared with offspring measures) because not all females became pregnant or produced four sons.

3. RESULTS

(a) *Physiological responses to treatment in the female*

Because no significant differences were detected among the four control treatments for the response
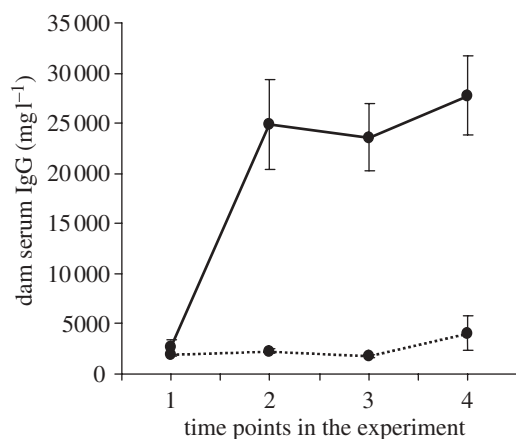
Figure 1. Female serum immunoglobulin, measured at the start of the experiment prior to treatment (time 1, day one), following clearance of infection and prior to mating (time 2, day 20), at the end of pregnancy (time 3, day 47) and upon weaning (time 4, day 60). Error bars represent $\pm$ 1 s.e. Filled circles with solid line, *Babesia* treatment; filled circles with dashed line, control treatments.

variables analysed (electronic supplementary material, table S1), these were grouped as one treatment labelled 'control treatments' and compared against the *Babesia*-treated females.

Following infection, *Babesia*-treated females showed significantly higher levels of serum IgG throughout the experiment than control females (figure 1, repeated measures generalized linear model (GLM) on $\log_{10}$ (IgG): $F_{3,105} = 39.983$, $p < 0.0001$). There was no effect of infection on serum corticosterone (repeated measures GLM: $F_{3,147} = 0.283$, $p = 0.838$). *Babesia*-treated females lost significantly more weight over the period of infection than females in all other control groups (GLM: $F_{1,72} = 7.517$, $p = 0.008$), but the difference in weight at the time of mating was of marginal significance (GLM: $F_{1,72} = 3.884$, $p = 0.053$). Body weight at mating did not appear to have significant downstream effects on the likelihood of becoming pregnant (GLM: $F_{1,72} = 1.272$, $p = 0.263$) or on litter size (Pearson's correlation: $n = 60$, $r = -0.203$, $p = 0.119$).

(b) *Reproductive responses to treatment in the females*
Infection with *B. microti* did not affect the likelihood of becoming pregnant (χ^2-test: $\chi^2 = 0.538$, d.f. = 1, $p = 0.970$) or producing a live litter (χ^2-test: $\chi^2 = 0.397$, d.f. = 1, $p = 0.983$). Nor did it affect the litter size (GLM: $F_{1,60} = 0.031$, $p = 0.831$) or the sex ratio of litters (GLM: $F_{1,57} = 0.056$, $p = 0.813$). However, the weight gain over pregnancy, and weight loss at parturition, were significantly affected by treatment. *Babesia*-infected females gained more weight during pregnancy (31.1 ± 0.56 g) and lost more at parturition (23.15 ± 0.61 g) than did control females (27.5 ± 0.63 and 20.9 ± 0.58 g, respectively), even when covariates were fitted to control for weight loss during infection and litter size (repeated measures GLM: $F_{2,116} = 6.566$, $p = 0.002$). *Babesia*-treated females then spent significantly more time nursing offspring than control females (figure 2a, GLM: $F_{1,51} = 5.940$, $p = 0.018$).

(c) *Responses to treatment in the young offspring*
It was not possible to look at the effects of treatment on the weight at birth in offspring, because interference with pups at this age carries too high a risk of infanticide. We detected no effect of maternal treatment on early pup growth (weight gain between day 11 and weaning; repeated measures GLM: $F_{2,114} = 0.818$, $p = 0.444$), but by weaning *Babesia*-treated pups were significantly lighter than control pups (figure 2b: GLM: $F_{1,51} = 4.914$, $p = 0.031$). This was particularly surprising as *Babesia*-treated pups spent significantly more time suckling (27.4 ± 0.83 scans compared with 22.62 ± 0.50 scans, GLM: $F_{1,51} = 6.806$, $p = 0.012$). The effect of treatment on weight was no longer evident by five weeks of age, or thereafter (repeated measures GLM: $F_{3,159} = 0.149$, $p = 0.930$).

Offspring from *Babesia*-treated females showed significantly higher levels of IgG at weaning at 24 days of age (1893 ± 307 mg l^{-1} compared with 1071.29 ± 147.6 mg l^{-1}; GLM with $\log_{10}$ transformed data: $F_{1,53} = 17.529$, $p < 0.001$), a difference that persisted to seven weeks of age (*Babesia*: 2139 ± 239 mg l^{-1}; control: 1450 ± 125 mg l^{-1}; GLM with $\log_{10}$ transformed data: $F_{1,53} = 9.612$, $p = 0.003$). There was no effect of treatment on early offspring corticosterone or testosterone (repeated measures GLMs: corticosterone: $F_{3,153} = 0.423$, $p = 0.737$; testosterone: $F_{3,159} = 0.647$, $p = 0.586$).

(d) *Responses to treatment in the adult offspring*
Over the social grouping phase, when the adult males met non-sibling conspecifics for the first time, offspring from control treatments showed an increase in testosterone production, whereas *Babesia*-treated males showed a reduction (figure 3; GLM of change in serum testosterone (after minus before): $F_{1,44} = 6.296$, $p = 0.016$). There was no such effect on corticosterone or immunoglobulin over the novel social group period (GLM of change in corticosterone: $F_{1,49} < 0.001$, $p = 0.993$; change in IgG: $F_{1,48} = 1.206$, $p = 0.278$). Despite the effect on testosterone, there was no observable effect of treatment on social behaviour in adult offspring (GLM of square-root transformed total number of observed acts of aggression: $F_{1,56} = 0.247$, $p = 0.622$).

(e) *Effect of treatment on the response of adult offspring to infection*
Offspring from *Babesia*-treated females had a significantly different response to *B. microti* infection as adults (repeated measures GLM: $F_{5,255} = 3.314$, $p = 0.006$) with a faster resolution phase of the infection profile (figure 4). The accelerated response to infection was reflected in *Babesia*-treated males losing less weight in the two week period after inoculation (1.57 ± 0.30 g lost by *Babesia*-treated males compared with 3.05 ± 0.22 g lost by controls; GLM: $F_{1,48} = 6.181$, $p = 0.016$). Interestingly, *Babesia*-treated males showed less of an increase in IgG over infection, even when early IgG levels were controlled for (*Babesia*: increase of $18\,038 \pm 2264$ mg l^{-1}; control:
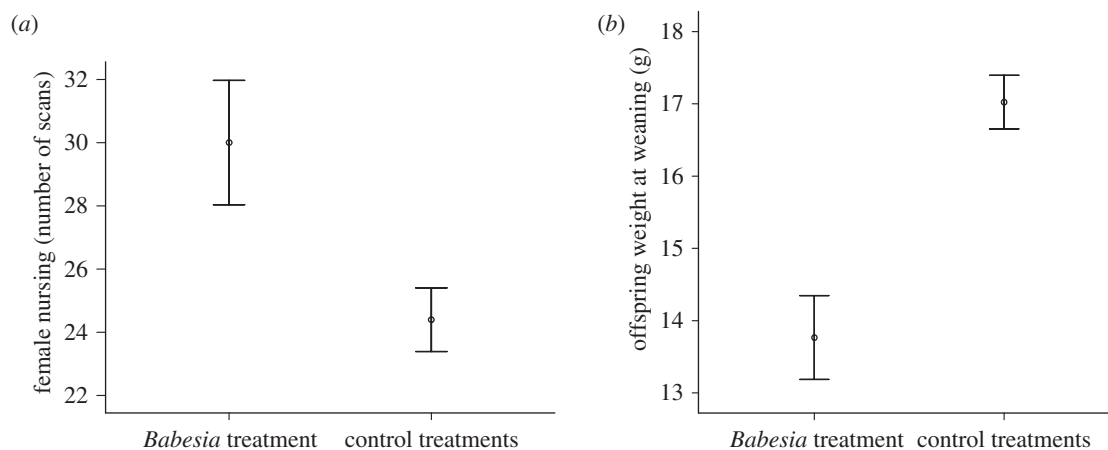
(a)

(b)

Figure 2. Female nursing behaviour and offspring growth. (*a*) Total number of instantaneous scans in which females were seen nursing one or more pup. (*b*) Offspring weight at weaning (24 days of age). Error bars represent $\pm$ 1 s.e.
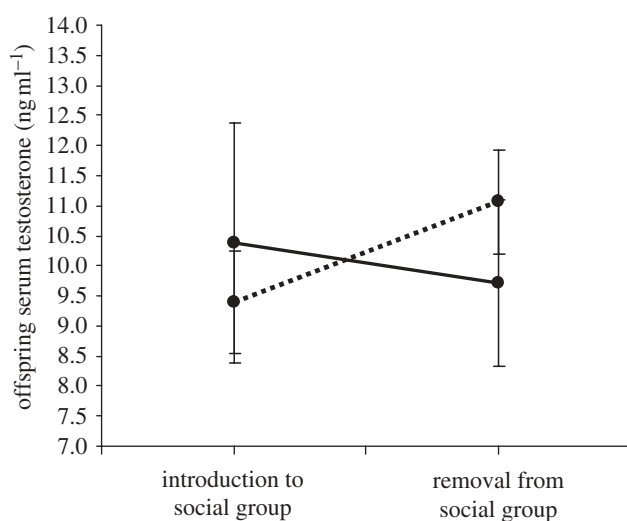

Figure 3. Changes in offspring testosterone. Serum testoster-one measured on introduction to (49 days old) and removal from (65 days of old) novel social group caging as adults. Error bars represent $\pm$ 1 s.e. Filled circles with solid line, *Babesia* treatment; filled circles with dashed line, control treatments.
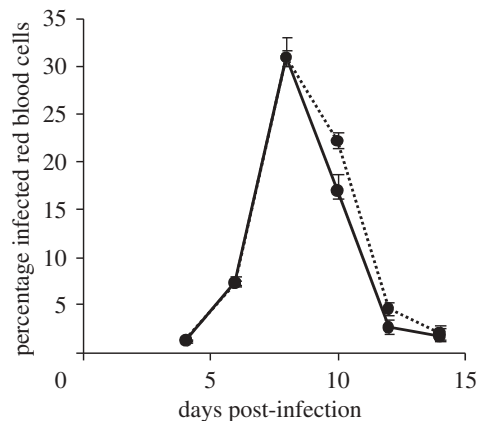

Figure 4. Offspring infection profiles. Percentage of red blood cells infected with *Babesia microti* at various time points following intraperitoneal injection with 1×10^7 infected red blood cells. Error bars represent $\pm$ 1 s.e. Filled circles with solid line, *Babesia* treatment; filled circles with dashed line, control treatments.
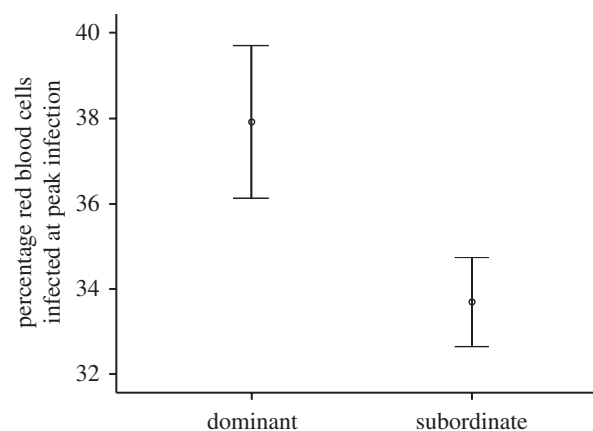
Figure 5. The effect of social rank on infection peaks. Off-spring grouped in cages of four were ranked as dominant (one per cage) or subordinate (three per cage) based on their aggressive behaviour. Graph shows subsequent infection levels at the peak of infection. Error bars represent $\pm$ 1 s.e.

$23\,826 \pm 2071$ mg l^{-1}; repeated measures GLM with log$_{10}$ transformed data: $F_{1,51} = 6.197$, $p = 0.016$).

There was no overall effect of social grouping on infection profile (repeated measures GLM: $F_{5,925} = 0.122$, $p = 0.988$), but males that were grouped and displayed the most aggression in their groupings (domin-ants) experienced higher infection peaks (figure 5: GLM: $F_{1,98} = 4.172$, $p = 0.044$) than males that were less aggressive in groupings (subordinates). Treatment did not interact with the effect of social rank on peak infection (GLM: $F_{1,96} = 0.004$, $p = 0.947$). Individuals of different rank did not show significantly different patterns of testosterone production over their life-times (repeated measures GLM: $F_{3,276} = 0.518$, $p = 0.671$). No significant interaction between rank and treatment on testosterone production over an individ-ual's lifetime was found (repeated measures GLM: $F_{3,270} = 1.883$, $p = 0.133$).

4. DISCUSSION
We examined how experience of recent infection affects reproductive output and maternal behaviour in female mice, and social behaviour and resistance to infection

in their offspring in subsequent adult life. The results support the findings of our previously reported study [51] in which females were indirectly exposed to infection by co-housing with non-contagious conspecifics. In both experiments, females exposed to immunological cues had physiological responses to their exposure, and produced offspring with an accelerated response to infection as adults. Unlike in the earlier study, here the sons of the directly infected females did not display lower aggression as adults, but nevertheless differed in their hormonal response to novel social environments, decreasing instead of increasing testosterone production upon social housing. Testosterone in male adult mice is associated with social dominance, aggression, territory acquisition and maintenance, and consequently increased access to mating opportunities [59–61]. However, there is evidence that the benefits of dominance are counterbalanced by costs associated with reduced resistance to infection [59,62,63]. Other studies strongly implicate testosterone in the mediation of these dominance–resistance trade-offs [32,59,62,64].

We believe that the patterns of maternal investment in *Babesia*-treated females in our study could reflect immunocompetence-directed investment. Females may be specifically priming offspring for life in an environment in which the risk of infection is high [9]. Our results add to a growing body of evidence that suggests that effects of this sort occur across a wide range of taxa. Queen bumblebees (*Bombus terrestris*) injected with bacteria produce workers that show higher levels of antibacterial activity [65]. Immunized pied flycatchers (*F. hypoleuca*) produce offspring with elevated endogenous antibody production [66]. Offspring from mice infected with an intestinal nematode when pregnant are more likely to clear infection as adults [20,67].

Although we observed a difference in hormonal response to social challenge in the offspring of *Babesia*-challenged females, we did not observe a concomitant difference in levels of aggression. This may have been because levels of observed aggression were generally low in our experiment, and also because none of the male offspring in this study had ever been singly housed prior to social grouping (single housing increases subsequent territorial behaviours; see [58]). Despite the low levels of aggression recorded, it was possible to allocate ranks within each cage group, and higher-ranking males showed a downstream immunocompetence cost, experiencing higher levels of infected cells at peak infection. This further supports the conclusion that a dominance–resistance trade-off occurred in this experiment.

We chose to study the effects of maternal infection on male offspring and not on females because a dominance–immunocompetence trade-off is relatively well documented in male mice [36–38,59], and because the inclusion of offspring of both sexes was not feasible while maintaining sample sizes at the levels that were indicated to be required by *a priori* power analysis. While our results are consistent with the idea of adaptive maternal mediation of a trade-off in male offspring, it is difficult to predict what might occur if our experimental observations were repeated with female offspring. Maternal effects on life-history traits and behaviour in the female offspring of rodents are well documented [68,69], but selection pressures on males and females are often very different, and it is generally expected that if mothers have the ability to adjust investment differentially in sons and daughters, maternal effects will be offspring-sex-specific [4,22]. While the upregulation of immunocompetence in sons is predicted to result in androgen-mediated changes in social behaviour/dominance, trade-offs with other behavioural or life-history traits are plausible in daughters. Litter and offspring body size, and the timing of birth, are known to be the target of maternal effects in female rodents [68,69], and we might therefore expect maternally induced redirection of resources towards immune defence to come at the expense of reduced fertility and fecundity, or modified reproductive behaviour. Until appropriate experiments are conducted in mice and other species, however, discussion of such differential maternal effects on the sexes in response to the threat of disease remains speculative.

Babesia-treated females gained more weight during pregnancy, lost more weight at parturition and spent more time nursing their offspring. This may reflect an increased investment in the current litter, to the potential detriment of future reproduction. Iteroparous species, such as mice, usually do not put all their investment into one reproductive bout, because it may reduce future reproductive success and therefore overall lifetime fecundity [70,71]. However, if longevity is reduced by a parasite, such a shift in investment priorities may occur [72]. Immunologically challenged common eider ducks (*Somateria mollissima*) incubate eggs for longer, lose more weight in the process and display higher levels of parental care after hatching [73], findings similar to those seen here. Nematode-infected pregnant mice produce significantly and substantially larger litters [20], but subsequent litters are smaller [74]. However, the similarity of these patterns is somewhat complicated by the unexpected finding that *Babesia*-treated offspring gained less weight than control offspring during the pre-weaning period. It is conceivable that increased suckling observed among the offspring of dams that had experienced infection was driven by the offspring rather than by the mothers, and hence may have reflected the need to increase suckling time owing to potentially limited or lower quality milk production [75–77]. An earlier experiment by Barnard *et al.* [37] showed that suckling behaviour affected both social rank and immunity in mice, with aggressiveness inversely proportional to suckling, but, as in our study, it was not possible to determine whether this was offspring- or parent-driven. The mice in the Barnard *et al.* study [37] that suckled more actually gained less weight, suggesting that increased suckling time does not reflect increased milk consumption [76]. These mice then developed into lower ranking individuals, but with enhanced immunocompetence [37]. The same relationship between suckling and weight gain was found in previous studies on mice [78]. In our experiment, *Babesia*-treated sons also suckled more, gained less weight and then went on to respond differently to both social and immunological challenges; it is thus possible that

suckling experience in infancy mediates immunological and social priming.

Our experiment appears to show a general increase in investment in the immune system in both mothers exposed to infection and their offspring. In our view, it is likely that such changes are adaptive for the mother and her offspring, equipping them better to face an environment in which the threat of infection is high. It is never possible, however, to exclude completely the chance that the responses seen are driven by adaptive manipulation of the host by the parasite, or indeed that the changes have no adaptive significance [24]. In our experiment, we allowed full clearance of infection before pregnancy, and it is unlikely that the direction of the responses observed in both females and offspring reflected increased fitness of *B. microti*. Instead, we believe that our findings support the conclusion that females invested in reproduction in such a way as to maximize offspring resistance at a time when the risk of disease was high.

We aimed to explore transgenerational effects of a parasitic infection in the mother, rather than foetal immunological responses to direct challenge. Therefore, it was important that offspring were not directly infected by vertical transmission [79]. Babesial agents have been shown on occasion to pass transplacentally from mother to offspring *in utero* [53,79]. For example, *Babesia bovis* in cattle, *Babesia canis* in dogs, *Theileria equi* (formerly *Babesia equi*) in horses and *B. microti* in humans have all been shown to pass through the placenta [53,79]. By infecting females prior to mating and ensuring that all infected females had cleared infection prior to mating, our experimental design was aimed at minimizing any risk of vertical transmission. We further validated that offspring were not infected by taking smears at weaning. Serum immunological constituents and response to infection later in life confirmed the absence of infection in newborn offspring (see §3).

Other than documented cases of vertical transmission, and foetal abortion owing to babesiosis during pregnancy [53], there is very little information in the literature regarding the impact of perinatal *Babesia* infection on offspring. More research has been done on the consequences of the similar malarial parasites, *Plasmodium falciparum* and *Plasmodium vivax*, which are very similar to *Babesia* spp., but which are a much more significant cause of serious disease and mortality in humans [80,81]. Research has shown that humans born to malarial mothers have lower birth weights owing to inter-uterine growth retardation. They also tend to be premature, and there is an associated increased risk of stillbirth (e.g. [82]). However, most of these studies of malaria investigate the implications of perinatal parasitaemia from a human health perspective. Very few offer insight into offspring life-history consequences of maternal infection (but see [81]), or into the potentially adaptive offspring disease resistance owing to maternal exposure prior to pregnancy. Here, we demonstrate a potentially adaptive life-history consequence for the offspring of infected mothers.

Following infection with *B. microti*, both females and their offspring showed elevated levels of IgG. In natural systems, *Babesia* species are transmitted into the host bloodstream by ticks [53], and IgG antibodies can block *B. microti* sporozoites from invading erythrocytes shortly following infection. If this fails, NK cells and macrophages act to limit the extent of parasitaemia [54]. Ultimately, a resolution stage begins, with parasitaemia levels peaking and then rapidly declining owing to the action of $CD4^+$ T cells and IFN-γ [54,56]. While IgG is primarily involved in the early stages of infection, it is expected that circulating levels remain high following clearance, and as such are a useful bystander measure of immunocompetence [36]. IgG is also of relevance to this study because it can pass between rodent females and their offspring via the milk [83]. Therefore, the elevated levels seen in the offspring of *Babesia*-treated females could be a direct consequence of changes occurring in the maternal immune system as a consequence of infection.

As adults, *Babesia*-treated offspring showed a faster resolution phase in response to direct *Babesia* infection. They also showed a relative reduction in testosterone in response to a social challenge. Testosterone is known to interact directly and indirectly with $CD4^+$ T cells (see [84] for a discussion of evidence of both up- and downregulation), which are important for the resolution phase of *B. microti* infection recovery [54]. The *Babesia*-treated offspring lost less weight during infection, indicating lower adverse effects from parasitism [85], possibly less pathology and hence lower intensity of disease. It is difficult to interpret the relatively low levels of IgG production during infection in this same treatment group. It may be that in *Babesia*-treated offspring, other immune cells (e.g. $CD4^+$ T cells) and molecules were downregulated less by testosterone, and therefore the *Babesia*-treated offspring were less dependent on escalating production and systemic sustenance of IgG. Alternatively, the difference may simply reflect a faster return to normal levels of immune components in the *Babesia*-treated offspring, because they cleared the infection more efficiently.

In conclusion, our results support the existence of a trade-off between social dominance and disease resistance, and suggest that such a trade-off can be mediated by maternal experiences before pregnancy. While our findings do not perfectly mimic those found following an indirect challenge by co-housing pregnant females with infected neighbours [51], consistent trends have emerged. In both cases, maternal exposure led to physiological changes in the female, which ultimately filtered down, affecting the responses of offspring to social challenge (either behaviourally or physiologically) and to infection. The presence of *Babesia* in the maternal environment caused offspring to downregulate investment in dominance acquisition, which in natural systems, among wild animals, would probably have led to reduced access to mating opportunities [59–61]. In doing so, however, the offspring avoided the associated reduced resistance to infection that is typically associated with high dominance status [59,62,86]. In an environment in which the risk of parasitism is high, cues experienced by the mothers led to increased resistance in offspring. The results of our experiment emphasize the roles of both maternal effects and parasites, and the interplay

between the two, in shaping life histories and disease susceptibility.

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