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Published in:
International Journal for Parasitology

DOI:
10.1016/j.ijpara.2014.12.005

Print publication: 01/01/2015

Document Version
Peer reviewed version

Tolerance and resistance to a nematode challenge are not always mutually exclusive

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Abstract

The relationship between the manifestations of tolerance (host's ability to reduce the impact of a given level of pathogens) and resistance (host's ability to clear pathogens) has been assumed to be an antagonistic one. Here we tested the hypothesis that mice from strains more resistant to intestinal nematodes will experience reduced tolerance compared to less resistant mice. Three inbred strains of mice were used: C57BL/6 mice have been characterised as susceptible, whereas BALB/c and NIH mice have been characterised as resistant to *Heligmosomoides bakeri* infection. Mice of each strain were either parasitized with a single dose of 250 L_3 *H. bakeri* (n=10) in water or were sham-infected with water (n=10). Body weight, food intake and worm egg output were recorded regularly throughout the experiment. Forty-two days post infection mice were euthanized and organ weights, eggs in colon and worm counts were determined. C57BL/6 mice showed significantly greater worm egg output ($P<0.001$), eggs in colon ($P<0.05$) and female worm fecundity ($P<0.05$) compared to NIH and BALB/c. Parasitized BALB/c mice grew more whilst parasitized C57BL/6 grew less than their sham-infected counterparts during the first two weeks post challenge ($P=0.05$). Parasitism significantly increased liver, spleen, small intestine and caecum weights ($P<0.001$), but reduced carcass weight ($P<0.01$). Average daily gain and worm numbers were positively correlated in NIH mice ($P=0.05$); however, the relationship was reversed when carcass weight was used as a measure for tolerance. BALB/c mice did not appear to suffer from the consequences of parasitism, with carcass weight similar in all animals. Our hypothesis that strains more resistant to the *H. bakeri* infection are less tolerant compared to less resistant strains is rejected, as the two resistant strains showed variable tolerance. Thus, resistance and tolerance to intestinal nematode infection are not always mutually exclusive.

47 Keywords: mice; genetic resistance; infection tolerance; nematodes; performance;

48 Heligmosomoides bakeri.

49

1. Introduction

The intestinal trichostrongyloid nematode *Heligmosomoides (polygyrus) bakeri* has been used as a model of chronic intestinal nematode infection for over four decades (Behnke et al., 2006). *H. bakeri* infection in mice induces a strongly polarized Th2 immune response, which has been shown to be critical for worm control and expulsion (Reynolds et al., 2012). The mechanisms underpinning helminth expulsion in mice are studied to facilitate predictions about the outcome of similar interactions between helminths and the immune system in livestock and humans, to enable the development of control strategies. The outcome of primary *H. bakeri* infection is strongly influenced by the genetic background of mice, with strains differing in their susceptibility to chronic infection (Reynolds et al., 2012). Whilst between-strain variation in nematode resistance has been previously described (Behnke et al., 2006), there is no evidence of desciption of variation in tolerance. Resistance describes the ability of the host to clear pathogens, whereas tolerance describes the ability to reduce the health or fitness impact of a given infection intensity (Ayres and Schneider, 2012; Raberg, 2014). Characterising the tolerance of mice strains that differ in their resistance to *H. bakeri* infection will facilitate the selection of the most appropriate mouse strain to model nematodiasis in human and livestock hosts.

The study of tolerance to parasites and its association with resistance has a long tradition in plant science, but very limited evidence is available from animals. Recently, the individual variation in tolerance to parasites has been described, in wild sheep and rodent populations (Hayward et al, 2014; Jackson et al, 2014). Haward et al (2014) observed a positive relationship between tolerance and evolutionary fitness in sheep, with more tolerant animals having higher lifetime breeding success. Jackson et al (2014) on the other hand observed a negative relationship between tolerance and reproduction, with more tolerant animals having reduced reproductive effort. The

association between the manifestations of tolerance and resistance to nematodes in animals has been previously assumed to be an antagonistic one (Doeschl-Wilson et al., 2009), although there is no experimental evidence to support this view. Råberg (2014) argued that when analysed simultaneously, tolerance and resistance should be under correlational selection; this implies either a negative relationship between these traits, or the possibility they may be both at intermediate levels.

Here we tested the hypothesis that between-strain variation in resistance to *H. bakeri* infection will correlate with between-strain variation in tolerance. The expectation was that mouse strains more resistant to the *H.bakeri* infection will be less tolerant compared to less resistant strains.

2. Materials and Methods

2.1. Experimental animals and housing

This animal experiment was approved by SRUC's Ethical Review Committee (ED AE 06/2011) and carried out under Home Office authorization (PPL 60/3626). A total of 60 5-wks-old male C57BL/6, NIH and BALB/c mice (n=20 per strain), were housed in a room with an ambient temperature of 21±1°C and a 12 h light cycle (07.00 to 19.00 h). Mice were individually housed in solid bottomed cages with fresh sawdust and bedding material provided weekly. Shredded paper was added as environmental enrichment. The three strains were selected based on variation in their susceptibility to infection with *H. bakeri* as defined by their phenotype during the infection. C57BL/6 mice have been characterised as poor responders to *H. bakeri*; they maintain a high worm burden that can persist for over 30 weeks (Behnke et al., 2006). NIH mice have been characterised as strong, early responders to *H. bakeri*; compared to C57BL/6 mice, worm

98 establishment is lower, and worm burdens are cleared out within seven weeks (Behnke *et al*.,
99 2006). BALB/c mice have also been characterised as strong responders, although worm expulsion
100 rate is slower than in NIH mice (Behnke et al., 2006; Reynolds et al., 2012).
101
102 *2.2. Infection protocol and experimental design*
103 The experiment was conducted over two consecutive blocks, balanced for all treatments, of 30
104 mice each. At day 0 of the experiment, mice of each strain received either a single dose of 250 *H.*
105 *bakeri* L_3 suspended in 0.2 ml of water (n=10) or a sham infection of 0.2 ml of water (n=10) via oral
106 gavage (Houdijk and Bunger, 2007). The *H. bakeri*, formerly known as *Heligmosomoides polygyrus*
107 *bakeri* and *Nematospiroides dubius* (Cable et al., 2006), were cultured from mono-specifically
108 infected donor mice. The dose of *H. bakeri* was chosen to produce a subclinical level of infection
109 that has been shown to affect mice growth (Houdijk and Bunger, 2006; 2007).
110
111 Mice were fed *ad libitum* throughout the experiment a maintenance diet (14% crude protein;
112 Special Diet Services, Lillico Biotechmologies, UK). Mice were monitored for 42 days post
113 nematode infection. On day 42 they were euthanized for sample recovery.
114
115 *2.3. Measurements and sample collection*
116 Body weight and food intake: Mice and food refusals were weighed three times weekly
117 throughout the experiment. On each of these days food refusals were weighed out and fresh food
118 added weighed in. From these measurements, food intake was calculated per mouse per day.
119 Food intake and body weight of mice were used as indicators of growth performance.
120

Nematode egg counts: Mice were placed onto wire-bottomed cages and faecal samples were collected on wetted cardboard on days 17, 24, 31, 38 post infection to assess faecal egg counts (eggs per g faeces). This was carried out using a modified flotation technique (Christie and Jackson, 1982). Faeces were collected over a 12h period, during which a constant rate of egg production was assumed. Egg output was expressed as eggs per 12h (EO) to account for possible dilution effect on faecal egg counts attributed to variable faecal outputs as a consequence of different food intake in different mice strains (Coltherd et al., 2009).

Internal organ weights, eggs in colon and worm burdens: On day 42 mice were humanely killed via CO2 inhalation and dissected for sample recovery. The small intestine was weighed, opened up and placed in a tube with PBS, which was then incubated at 37°C for 3 h to allow worms to migrate out of the tissue. Tissue and recovered worms were stored in a 5% formalin solution. Male and female worms were separated and counted. The colon contents were weighed and an egg count was performed with the same floatation technique (Christie and Jackson 1982). The colon egg count was then multiplied by colon contents weight to account for dilution effects arising from variation in food intake and colon content volumes between the different strains. Resultant data were expressed as number of eggs in colon (EIC, number of eggs). The EIC was divided by the number of females counted to obtain an estimate for the *per capita* fecundity (eggs per female). EO, EIC, *per capita* fecundity and total worm counts were used to confirm variation in resistance of the strains. EIC and worm counts were used in tolerance estimates, as explained below.

Measures of tolerance: Individual tolerance was estimated in two different ways. Firstly, we associated carcass weight at dissection (true reflection of performance) and worm burdens recovered at dissection (accurate estimate of parasite load). We also associated average daily gain, which is often used as an indirect indicator for performance and eggs in colon (EIC), as an indirect

indicator for parasite load. Our expectation was that tolerance estimates will be similar in both cases.

2.4. Statistical analysis

Data were analysed in Genstat 11th Edition (VSN International LTD, 2008). Model assumptions were tested on normality of means and residuals. Average daily body weight gain and food intake during worm establishment (P1: days 0-16) and the established infection (P2: days 17-42) were analysed through a 3 x 2 factorial ANOVA (3 strains x 2 levels of parasitism) with pre-infection body weight used as covariate. Carcass and internal organ weights were analysed through the same 3 x 2 factorial ANOVA, again with pre-infection body weight as covariate. EIC, EO, worm counts and worm fecundity were analysed in parasitised animals by one-way ANOVA (3 strains). The EO data were analysed in a repeated measure model. Due to the skewed nature of the data, EO, EIC and *per capita* fecundity were log10 (n) transformed. These are reported as back-transformed least-square means, accompanied by lower and upper confidence intervals, calculated from back-transforming least-square mean of transformed data (m), m–S.E. and m+S.E respectively. Experimental round was included in both statistical models as block. Effects with P-values less than 0.05 are considered significant whilst those with P-values between 0.05 and 0.10 are described as tendencies or trends. Pearson's correlations were performed between aforementioned *a priori* selected parasitological and performance data to characterise the tolerance of different mice strains to the *H. bakeri* infection.

3. Results

3.1. H. bakeri nematodes were more prolific in C57BL/6 than in NIH or BALB/c mice

There was a significant strain effect on EO (P<0.001) with C57BL/6 mice excreting more eggs compared to NIH and BALB/c. EO eggs/12h reached 48,000 (39,000-60,000) in C57BL/6 mice, 25,000 (20,000-31,000) in BALB/c mice and 24,000 (20,000-30,000) in NIH mice, day 38 post challenge. There was a significant time effect, with EO increasing over time in all strains (P=0.004). The strain x time interaction was also significant, with the rate of increase being greater in C57BL/6 mice compared to mice from the other strains (Figure 1).

<<Figure 1 here>>

EIC determined on day 42 was significantly affected by strain at the same direction as EO (P=0.033); C57BL/6 mice had significantly greater EIC compared to mice of the other two strains (Figure 2).

<<Figure 2 here>>

There was no significant strain effect on total (P=0.189), male (P=0.239) or female (P=0.156) worm counts, although worm burdens were 40% greater in C57BL/6 mice compared to NIH (Figure 3). Strain significantly affected *per capita* fecundity (P=0.045), which averaged 535 (475-604), 338 (275-415) and 280 (230-341) eggs/female worm in C57BL/6, BALB/c and NIH mice, respectively.

<<Figure 3 here>>

3.2. The impact of parasitism on performance was short-lived and strain-dependant

Infection reduced food intake in each strain during the first 16 days post challenge (P1); across strains, food intake of parasitized and sham-infected mice averaged 4.23 and 4.64 g/day, respectively ($P<0.001$; Table 1). NIH mice consumed the most and C57BL/6 consumed the least food ($P<0.001$). Between days 17-42 post infection (P2), the strain effect on food intake was sustained with C57BL/6 mice consistently eating the least and NIH mice eating the most food ($P<0.001$). However, the effect of parasitism disappeared during this period; across strains, food intake of parasitized and sham-infected mice averaged 4.40 and 4.32 g/day, respectively.

<<Table 1 here>>

During P1, body weight gain did not differ between parasitized and sham-infected mice across strains ($P=0.540$), whilst NIH mice grew faster than C57BL/6 mice, with BALB/c being intermediate ($P<0.001$; Table 1). However, an interaction demonstrated that parasitized BALB/c mice grew more and parasitized C57BL/6 grew less than their sham-infected counterparts ($P=0.056$). During P2, there was no significant difference in body weight gain between parasitized and sham-infected mice ($P=0.432$). The strain effect on body weight gain remained significant, with NIH mice growing faster compared to C57BL/6 and BALB/c mice ($P<0.001$).

3.3. Parasitism and mouse strain had an impact on internal organs weight

Strain significantly affected the weight of most internal organs measured (Table 2). The weight of liver, spleen, small intestine and caecum were significantly greater in parasitized mice compared to non-parasitized ones ($P<0.01$). However, the interaction between parasitism and strain was significant for caecum and spleen weight; the caecum weighed significantly more in parasitized C57BL/6 mice ($P=0.030$) compared to parasitized mice from the other strains, whereas the spleen

of parasitized NIH mice weighed significantly more compared to parasitized mice from the other strains ($P<0.001$). Across strains, spleen weight was negatively correlated with total number of worms recovered and with EIC; mice with heavier spleens had fewer worms ($r=-0.46$; $P=0.01$) and EIC ($r=-0.63$; $P=0.001$)

Final body weight at dissection was affected by strain; NIH mice weighed the most and C57BL/6 the least, with BALB/c weighing intermediate ($P=0.028$). Carcass weight followed a similar pattern across strains ($P<0.001$). Parasitism did not affect the final BW of mice but significantly reduced carcass weight ($P=0.003$).

<<Table 2 here>>

<<Table 3 here>>

3.4. Using body weight gain to estimate tolerance may underestimate the impact of parasitism on performance

Table 3 shows that for NIH mice only, there was a significant positive correlation between averaged daily gain (ADG) and worm numbers ($r=0.62$; $P=0.05$). However, when carcass weight was used to calculate tolerance, this relationship was reversed; the greater the number of worms recovered at dissections, the lower the carcass weight of NIH mice ($r=-0.48$; $P>0.05$). Although the correlations between carcass weight and worm counts in BALB/c mice were not significant, BALB/c mice appear to be the least affected by the infection compared to the other strains.

4. Discussion

238 This is the first study, where the tolerance to a nematode infection was quantified in strains of
239 mice that differ in their degree of resistance to *H. bakeri*. Three major outcomes were delivered
240 from the study. Firstly, we demonstrated that resistance and tolerance to a nematode parasite
241 infection are not necessarily mutually exclusive. The resistant BALB/c mice appear to be more
242 tolerant and least affected by the infection compared to the other strains. Secondly, we
243 demonstrated variation in tolerance between different strains of mice, which emphasizes the
244 importance of selecting the appropriate strain as a model of chronic nematode infection in
245 different mammalian hosts. Thirdly, we have clearly shown that body weight gain may not be the
246 best performance indicator to estimate the tolerance to parasitic challenge. Carcass
247 measurements revealed that the impact of parasitism was underestimated when based on body
248 weight gain measurement, as a consequence of an increase in the weight of internal organs in
249 parasitized mice, including spleen, liver, small intestine and caecum.
250
251 We used four measurements, namely EO, EIC, *per capita* fecundity and total worm counts to
252 confirm variation in resistance, i.e. the ability of the host to clear pathogens, of three mice strains.
253 Throughout the experiment, C57BL/6 mice excreted the largest number of eggs, compared to mice
254 from the other strains. Similarly, estimates of EIC and fecundity at dissection confirmed that
255 C57BL/6 mice were the most susceptible to the infection, as previously reported (Behnke et al,
256 2006). The genetic factors controlling resistance to *H. bakeri* include the major histocompatibility
257 complex (MHC) H-2 loci with C57BL/6 mice categorised as susceptible and BALB/c and NIH mice as
258 resistant genotypes (Reynolds et al, 2012). During trickle infections, differences in worm burdens
259 are evident within 6 weeks of the first challenge (Benkhe et al, 2006). However, following a single
260 challenge worm expulsion starts after week 10 post infections (Robinson et al, 1989), which would

be consistent with the similar worm numbers at 6 weeks post infection in all mice strains in our experiment.

To estimate tolerance in the three strains of mice, body weight throughout the experiment and carcass at dissections were taken. This detailed monitoring revealed that using carcass weight to estimate tolerance may be the most appropriate indicator of animal performance. Parasitism reduced carcass weight by an average of 5%, an effect that was not evident from body weight monitoring. This was due to increases in internal organ weight in parasitized mice, namely the small intestine, caecum, liver and spleen, which masked the penalties of parasitism on performance. Body weight gain measurements alone showed that parasitism did not penalize performance of NIH and C57BL/6 mice, whereas parasitized BALB/c mice appeared to grow faster than their sham-infected counterparts. Our findings strongly suggest that the well established negative impact of gastrointestinal nematode infections on animal performance, as measured by change in body weight (e.g. Sykes 1997) may be underestimated.

The increase in the weight of the small intestine observed in parasitized mice is in agreement with previous studies (Wong et al., 2007). This may be related to increased local inflammatory responses (Cywiniska et al 2004), plasma extravasation and mucin production as a consequence of parasitism (Wakelin, 1978). Furthermore, crypt hypertrophy and an increase in villi height has been observed in mice infected with *H. polygyrus*; it has been hypothesised that this response is adaptive to increase mucosal surface, to maintain nutrient absorption in the presence of the nematodes (Wong et al., 2007). Spleen size was significantly increased in parasitized mice; spleen enlargement has been observed during infections with filarial and gastrointestinal nematodes in variety of hosts (John, 1994; Wong et al., 2007). This increase in size is likely the direct outcome of

splenic cell proliferation (Katona et al., 1983), which reflects host resistance. Although spleen size was increased in all parasitized animals, the increase was greater in the resistant NIH mice, which is in agreement with previous evidence (Ali and Behnke, 1985). *H. bakeri* infection also affected liver weight in all strains. Hepatomegaly has been previously associated with nematode infection, particularly during the visceral migration of the larvae in species such as *Toxocara canis* (Pecinali et al., 2005) and in certain strains of mice during *H. bakeri* infection (Wong et al., 2007). It has been shown that seven days post a *H. polygyrus* infection liver cytokines, including pro-inflammatory cytokines, were up-regulated, in the absence of pathological and inflammatory response (Helmby, 2009). This early up-regulation of pro-inflammatory cytokines, such as IFN-ɣ, may be responsible for a liver inflammation at later stages of infection, which may result in hepatomegaly, as this was observed in our study.

Our hypothesis was that strains more resistant to the *H. bakeri* infection are less tolerant compared to less resistant strains. As a consequence of the detailed monitoring of host's performance, it became apparent that the two resistant strains varied in their tolerance levels (Table 3), with BALB/c more tolerant than NIH mice and thus the hypothesis is rejected. This supports the view that disease resistance and tolerance are not always mutually exclusive, but they may both be at intermediate levels if this promotes evolutionary fitness (Råberg, 2014). The mechanistic basis of this relationship is still to be determined. The study has also clearly characterised the variation in tolerance to *H. bakeri* of different strains of mice. Although it is unclear at this stage what the underlying basis of this variation was, it ought to be considered to inform on model choice for chronic nematode infection in human and animal hosts. BALB/c mice do not appear to be a good model for livestock, as livestock do not tolerate well nematode challenge, which is evident from the penalties on performance within weeks from the onset of an

infection (Coop et al, 1982). However, in humans, nematode parasitic gastroenteritis may be asymptomatic and is often not manifested with changes in body weight or body weight gain (http://emedicine.medscape.com/article/224011-clinical). Therefore, BALB/c mice may be a more appropriate model for parasitic gastroenteritis in humans, whilst C57BL/6 mice may be the better model for livestock.

Acknowledgements

This work was supported by the UK Biotechnology and Biological Sciences Research Council (BBRSC), Department for International Development (DFID) and Scottish Government (SG) under the umbrella of their CIDLID initiative (BB/H009299/1). We are thankful to Julie van Mierlo, Thom van Overbeek and Sandra Terry for their technical support. We are also thankful to Ian Nevison from Biomathematics and Statistics Scotland for statistical advice.

References

Ali, N.M.H., Behnke, J.M., 1985. Observations on the gross changes in the secondary lymphoid organs of mice infected with *Nematospiroides dubius.* J. Helminthol. 59, 167- 174.

Ayres, J.S., Schneider, D.S., 2012. Tolerance of Infections. Annu. Rev. Immunol. 30, 271–294.

Behnke, J. M., Mugambi, J. M., Clifford, S., Iraqi, F. A., Baker, R. L., Gibson, J. P., Wakelin, D., 2006. Genetic variation in resistance to repeated infections with *Heligmosomoides polygyrus bakeri*, in inbred mouse strains selected for the mouse genome project. Parasite Immunol. 28, 85-94.

Cable, J., Harris, P.D., Lewis, J.W., Behnke, J.M., 2006. Molecular evidence that *Heligmosomoides polygyrus* from laboratory mice and wood mice are separate species. Parasitology 133, 111–122.

Christie, M., Jackson, F., 1982. Specific identification of Strongyle eggs in small samples of sheep faeces. Res. Vet. Sci. 32, 113–117.

Coltherd, J.C., Bünger, L., Kyriazakis, I., Houdijk, J.G.M., 2009. Genetic growth potential interacts with nutrition on the ability of mice to cope with *Heligmosomoides bakeri* infection. Parasitology 136, 1043-1055.

Coop, R.L., Sykes, A.R., Angus, K.W., 1982. The effect of three levels of intake of *Ostertagia circumcincta* larvae on growth rate, food intake and body composition of growing lambs. J. Agric. Sci. 98, 247–255.

Cywińska, A., Czumińska, K., Schollenberger, A., 2004. Granulomatous inflammation during *Heligmosomoides polygyrus* primary infections in FVB mice. J. Helminthol. 78, 17-24.

Doeschl-Wilson, A.B., Brindle, W., Emmans, G., Kyriazakis, I., 2009. Unravelling the relationship between animal growth and immune response during micro-parasitic infections. PLoS One. 19, e7508. doi: 10.1371/journal.pone.0007508.

Hayward, A.D, Nussey, D.H., Wilson, A.J., Berenos, C., Pilkington, J.G., Watt, K.A., Pemberton, J.M., Graham, A.L., 2014. Natural selection on individual variation in tolerance of gastrointestinal nematode infection. PLoS Biology 12 (7), e1001947

Helmby, H., 2009. Gastrointestinal nematode infection J. Immunol. 182, 5663-5671.

Houdijk, J.G.M., Bünger, L., 2006. Selection for growth increases the penalty of parasitism on growth performance in mice. Proc Nut Soc 65, 68A.

354 Houdijk, J. G. M., Bünger, L., 2007. Interactive effects of selection for growth and protein supply on
355 the consequences of gastrointestinal parasitism on growth performance in mice. Proc Brit Soc
356 Anim Sci, pp 92.
357 Jackson, J.A., Hall, A.J., Ralli, C., Lowe, A., Zawadzka, M., Turner, A.K., Stewart, A., Birtles, R.J.,
358 Paterson, S., Brsdlay, J.E., Begon, M., 2014. An immunological marker of tolerance to infection
359 in wild rodents. PLoS Biology, 12(7), e1001901.
360 John, J.L., 1994. Nematodes and the spleen: an immunological relationship. Experientia (Basel), 50,
361 15–22.
362 Katona, I.M., Urban, J.F. Jr, Scher, I., Kanellopoulos-Langevin, C., Finkelman, F.D. 1983. Induction of
363 an IgE response in mice by *Nippostrongylus brasiliensis*: characterization of lymphoid cells with
364 intracytoplasmic or surface IgE. J. Immunol. 130, 350-356.
365 Pecinali, N.R., Gomes, R.N., Amendoeira, F.C., Pereira Bastos, A.C.M., Martins, M.J.Q.A., Pegado,
366 C.S., Pereira Bastos, O.M., Bozza, P.T., Castro-Faria-Neto, H.C., 2005. Influence of murine
367 *Toxocara canis* infection on plasma and bronchoalveolar lavage fluid eosinophil numbers and its
368 correlation with cytokine levels. Vet. Parasitol. 134, 121-130.
369 Raberg, L., 2014. How to live with the enemy: understanding tolerance to parasites. PLoS Biology,
370 12 (11), e1001989.
371 Reynolds, L.A., Filbey, K.J., Maizels, R.M., 2012. Immunity to the model intestinal helminth parasite
372 *Heligmosomoides polygyrus*. Seminars in Immunopathology 34, 829-846.
373 Robinson, M., Wahid, F., Behnke, J.M., Gilbert, F.S., 1989. Immunological relationships during
374 primary infection with *Heligmosomoides polygyrus* (*Nematospiroides dubius*): dose-dependent
375 expulsion of adult worms. Parasitology 98, 115-124.

376 Sykes, A.R., 1997. Effects of nematode parasitism on ruminant performance. In: Sustainable
377 Control of Internal Parasites in Ruminants: Animal Industries Workshop, Barrell GK (ed),
378 Canterbury, New Zealand, Lincoln University, pp. 81–92.
379 Wakelin, D., 1978. Genetic control of susceptibility and resistance to parasitic infection. Adv.
380 Parasitol. 16 , 219–308.
381 Wong, T., Hildebrandt, M., Thrasher, S.M., Appleton, J.A., Ahima, R.S., Wu, G.D., 2007. Divergent
382 metabolic adaptations to intestinal parasitic nematode infection in mice susceptible or resistant
383 to obesity. Gastroenterology. 133, 1979–1988.
384
385

Legends to Figures

Figure 1. Backtransformed means of egg output (EO) of mice infected with 250 *Heligmosomoides bakeri* infective larvae. Mice were one of three strains (n=10): C57BL/6, NIH, or BALB/c, which have been characterised as susceptible or resistant to *H. bakeri* infection. Lower and upper error bar values are backtransformed values of log-transformed mean minus or plus its standard error, respectively.

Figure 2. Backtransformed means of colon egg counts (CEC) of mice infected with 250 *Heligmosomoides bakeri* infective larvae. Mice were one of three strains (n=10): C57BL/6, NIH, or BALB/c, which have been characterised as susceptible or resistant to *H. bakeri* infection. Lower and upper error bar values are backtransformed values of log-transformed mean minus or plus its standard error, respectively.

Figure 3. Backtransformed means of adult male, adult female, and total worm burdens of mice infected with 250 *Heligmosomoides bakeri* infective larvae. Mice were one of three strains (n=10): C57BL/6, NIH, or BALB/c, which have been characterised as susceptible or resistant to *H.bakeri* infection. Lower and upper error bar values are backtransformed values of log-transformed mean minus or plus its standard error, respectively.

Figure 1

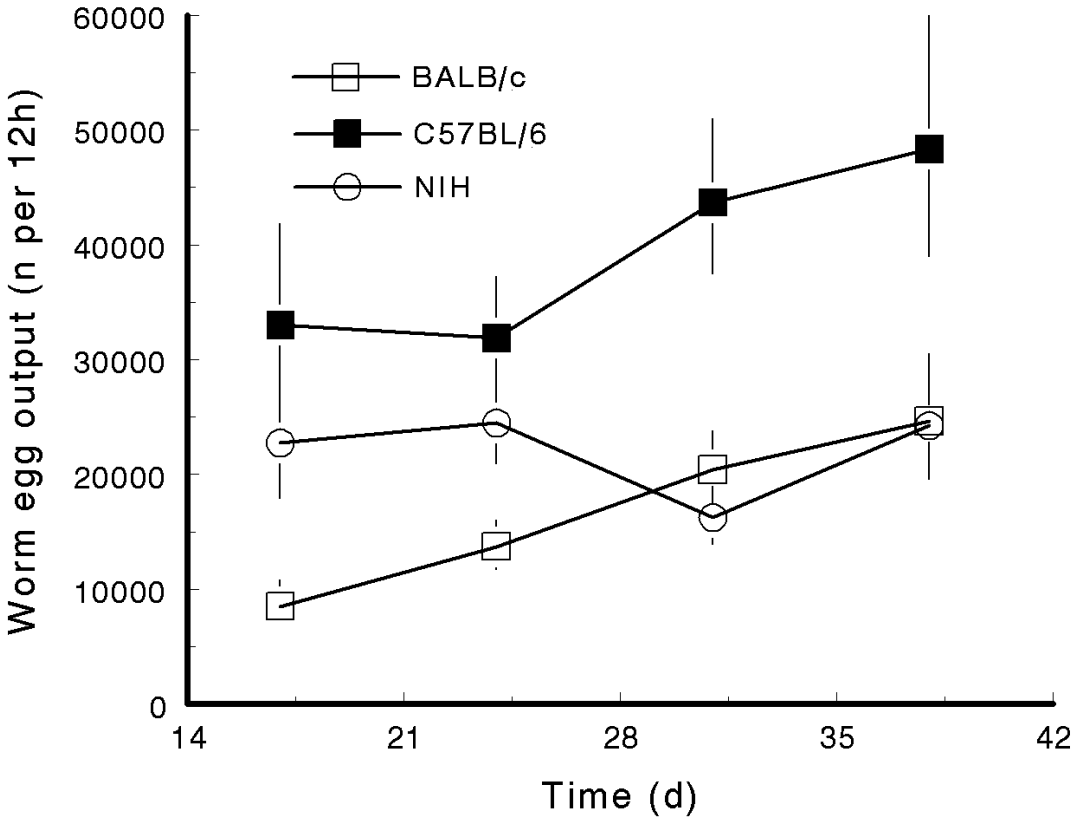
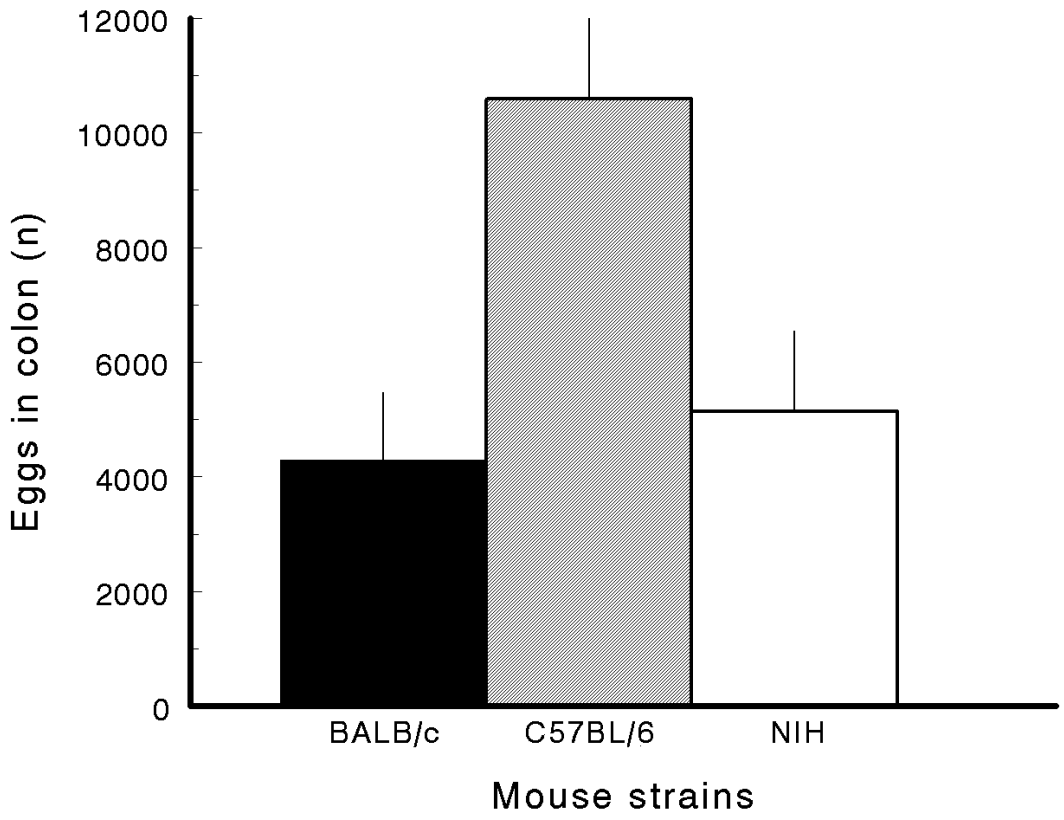

60000
50000
40000
30000
20000
10000
0
Worm egg output (n per 12h)
BALB/c
C57BL/6
NIH
14
21
28
35
42
Time (d)

Figure 2


12000
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8000
6000
4000
2000
0
Eggs in colon (n)
BALB/c
C57BL/6
NIH
Mouse strains

Figure 3

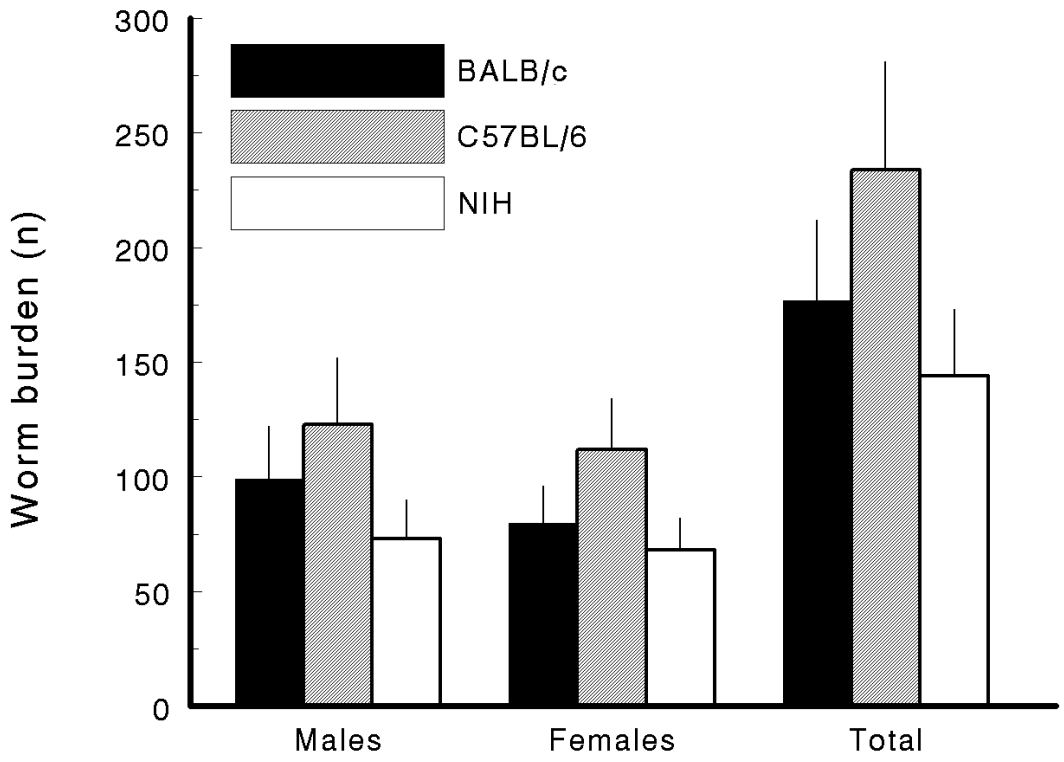
300
250
200
150
100
50
0
Worm burden (n)
BALB/c
C57BL/6
NIH
Males
Females
Total

Table 1. Mean voluntary food intake (VFI, g/day) and average daily gain (ADG, g/day) of C57BL/6, NIH or BALB/c mice over 42 days after infection with 250 *Heligmosomoides bakeri* infective larvae in 0.2 ml water (Par) or sham-infected with 0.2 ml water (Sham).

		Period 1[1]		Period 2[1]	
Strain	Treatment	VFI	ADG	VFI	ADG
BALB/c	Par	4.18	0.271	4.52	0.152
	Sham	4.56	0.187	4.46	0.115
C57BL/6	Par	3.84	0.130	3.71	0.097
	Sham	4.01	0.169	3.69	0.117
NIH	Par	4.84	0.359	4.97	0.157
	Sham	5.31	0.365	4.80	0.151
	S.E.D.[2]	0.095	0.036	0.114	0.021
Significance (P values)[3]					
Strain		<0.001	<0.001	<0.001	0.009
Parasitism		<0.001	0.540	0.149	0.432
Strain × Parasitism		0.179	0.056	0.579	0.126

[1]Period 1 relates to nematode establishment (day 0 – 16), whereas Period 2 relates to having a potent infection (day 1-42)

[2]Standard error of the differences between the means for the interaction term (n=10)

[3]The effect of the block was not significant and thus results are reported across the two blocks

Table 2. Final body weight, carcass, viscera and spleen weights (g) of C57BL/6, NIH or BALB/c mice, 42 days after infection with 250 *Heligmosomoides bakeri* infective larvae in 0.2 ml water (Par) or sham-infected with 0.2 ml water (Sham).

Strain	Treatment	BW[1]	Carcass	Stomach	SI[2]	LI[3]	Caecum	Liver	Spleen
BALB/c	Par	29.1	21.5	0.34	2.83	0.35	0.68	1.50	0.138
	Sham	27.1	21.7	0.39	1.59	0.32	0.51	1.32	0.101
C57BL/6	Par	25.3	18.9	0.39	2.73	0.24	0.92	1.23	0.097
	Sham	25.4	20.8	0.32	1.45	0.21	0.59	1.11	0.067
NIH	Par	32.6	24.8	0.57	3.07	0.32	0.68	1.71	0.195
	Sham	32.3	26.2	0.48	1.93	0.29	0.63	1.58	0.113
	S.E.D.[4]	0.90	0.68	0.093	0.121	0.040	0.073	0.075	0.006
Significance (P-values)[5]									
Strain		<0.001	<0.001	0.022	<0.001	0.002	0.020	<0.001	<0.001
Parasitism		0.166	0.003	0.475	<0.001	0.258	<0.001	0.002	<0.001
Strain × Parasitism		0.214	0.168	0.503	0.679	0.966	0.030	0.792	<0.001

[1]Final body weight at post mortems

[2]Small Intestine with content

[3]Large Instestine without content

[4]Standard error of the differences between the means for the interaction term (n=10)

[5]The effect of the block was not significant and thus results are reported across the two blocks.

Table 3. Pearson's correlations[1] between worm counts or the number of worm eggs in the colon contents (EIC), and average daily body weight gain (ADG), carcass and spleen weight in selected mice strains (n=10).

		BALB/c	C57BL/6	NIH
Worms	ADG	0.22	0.12	0.62*
	Carcass	0.37	-0.08	-0.48
	Spleen	-0.35	-0.62*	-0.49
EIC	ADG	0.30	-0.31	0.33
	Carcass	0.50	-0.06	-0.15
	Spleen	0.16	-0.36	-0.50

[1]Superscripts denote significance of r at $P<0.05$ (*)