Published in final edited form as:
Parasitology. 2009 April ; 136(5): 567–577. doi:10.1017/S0031182009005617.

The effect of infection history on the fitness of the gastrointestinal nematode *Strongyloides ratti*

Colin Bleay[1], **Clare P. Wilkes**[1], **Steve Paterson**[2], and **Mark E. Viney**[1]

[1]School of Biological Sciences, University of Bristol, Woodland Road, Bristol, BS8 1UG, UK

[2]School of Biological Sciences, University of Liverpool, Liverpool, L69 7ZB, UK

Summary

Hosts in nature will often acquire infections by different helminth species over their lifetime. This presents the potential for new infections to be affected (particularly *via* the host immune response) by a host's history of previous con- or hetero-specific infection. Here we have used an experimental rat model to investigate the consequences of a history of primary infection with either *Nippostrongylus brasiliensis*, *Strongyloides venezuelensis* or *S. ratti* on the fitness of, and immunological response to, secondary infections of *S. ratti*. We found that a history of con-specific, but not hetero-specific, infection reduced the survivorship of *S. ratti*; the fecundity of *S. ratti* was not affected by a history of either con- or hetero-specific infections. We also found that a history of con-specific infection promoted Th2-type responses, as shown by increased concentrations of total IgE, *S. ratti*-specific IgG_1, rat mast cell protease II (RMCPII), IL4 (but decreased concentrations of IFNγ) produced by mesenteric lymph nodes (MLNs) in response to *S. ratti* antigen. Additionally, *S. ratti*-specific IgG_1 was positively related to the intensity of both primary and secondary infections of *S. ratti*. Hetero-specific primary infections were only observed to affect the concentration of total IgE and RMCPII. The overall conclusion of these experiments is that the major immunological effect acting against an infection is induced by the infection itself and that there is little effect of prior infections of the host.

Keywords

density-dependence; parasite interactions; immuno-epidemiology; immuno-ecology

Introduction

In nature, hosts are usually exposed to infection by a range of infectious organisms, including different species of gastrointestinal helminths (Behnke, 2008). Yet, laboratory studies typically analyse only single species infections. While such analyses have given a good understanding of the biology and host immunology of specific infections, studies of single infections are not able to investigate how this is affected by infection with other species.

Interactions between co-infecting species may be direct, where two parasite species compete for a limited resource such as food or space within the host. Interactions between parasite species may also be indirect, particularly where they are mediated via the host immune response (Keymer, 1982). Thus, parasites do not need to infect at the same site within a host to interact if immune cross-reactivity (via either immune initiators or effectors) occurs

To whom correspondence should be addressed: Mark Viney, Email: Mark.Viney@bristol.ac.uk, 'phone: +44 (0)117 928 7469, Fax: +44 (0)117 331 7985 .

between them. Similarly, an infection may be affected by the host's previous infection history where immunological memory acts to mediate indirect interactions between parasites.

Helminth parasites cause a characteristic T-helper 2 (Th2)-style immune response, in contrast to most micro parasites that result in a Th1-style immune response (Finkelman *et al.*, 1997). In view of this, there may be greater potential for immunologically-mediated indirect interactions between different helminth species compared with potential interactions between helminths and non-helminths.

Previously, we have found that the gastrointestinal nematode *Strongyloides ratti* is subject to immune-dependent density-dependent effects (Paterson and Viney 2002). Thus, the probability of survival and the fecundity of individual parasitic female worms is negatively affected by the density of co-infecting con-specifics, with this effect dependent on the host immune response. We have also found that the host anti-*S. ratti* immune response is qualitatively and quantitatively affected by the density of infection (Bleay *et al.*, 2007), moving from a Th1 to a Th2 type profile with dose. This suggests that this dose-dependent qualitative and quantitative change of the host immune response causes the density-dependent effects on components of fitness of *S. ratti.* We also found that these immune-dependent effects could occur *via* immunological memory (Paterson and Viney, 2002). Previous studies have shown the possibility of hetero-specific effects on *S. ratti* infections. Thus, immunisation with a comparatively high dose (*e.g.* 3,000 larvae) of *Nippostrongylus brasiliensis* significantly reduced the number of worms recovered from a secondary *S. ratti* infection (Nawa *et al.*, 1982). Similarly, a primary *Trichinella spiralis* infection resulted in reduced survival of a subsequent *S. ratti* infection (Moqbel and Wakelin, 1979). The development of the *S. ratti* free-living generation of *S. ratti* is affected by the anti-*S. ratti* immune response (Harvey *et al.,* 2000). However, hetero-specific host immunisation also affects this development, though to a lesser degree than con-specific immunisation (West *et al.*, 2001).

We wished to determine the extent to which a history of either con- or hetero-specific nematode infection affects the fitness of, and immunological response to, subsequent *S. ratti* infection. We hypothesise that prior infection of the host will (i) negatively affect subsequent *S. ratti* infection, (ii) enhance the host anti-*S. ratti* immune response, (iii) that both these effects will be comparatively greater for prior infection with species that are most closely related to *S. ratti*, and (iv) that both these effects will be comparatively greater in more high intensity prior infections.

Methods

Parasites and experimental design

The *S. ratti* isofemale line ED321 Heterogonic was used throughout (Viney, 1996). A strain of *S. venezuelensis* was obtained from H. Maruyama (Nagoya City University Medical School) and a strain of *Nippostrongylus brasiliensis* was obtained from R.M. Maizels (University of Edinburgh). These three parasite species were maintained by serial passage in Wistar rats.

The experimental design was to infect rats with different doses of one of three gastrointestinal nematodes (*S. ratti, S. venezuelensis* or *N. brasiliensis*), to clear these infections and to subsequently infect the rats with different doses of an *S. ratti* infection. In this way, the effect of prior infection (both species and dose) on components of fitness of the secondary *S. ratti* infection and on the hosts' immune response could be investigated. The overall experimental design is shown in Table 1. Eighty four female Wistar rats of *c.*100g weight were randomly assigned among these 21 treatments (*i.e.*, 4 rats per treatment), with the experiment conducted in two equal experimental blocks (*i.e.*, 2 rats per treatment per experimental block), with the blocks separated by 28 days. The primary infection was given on day 0 post infection (p.i.) by the subcutaneous administration of infective third stage larvae (iL3s), as previously described (Wilkes *et al.*, 2007); control animals were given a sham inoculation of PBS only. Faeces were collected on days 5, 8 and 12 p.i., cultured appropriately to the infecting parasite species (Table 1). For *Strongyloides* spp. this is as described previously (Viney, 1996); for *N. brasiliensis*, host faeces were mixed with an equal volume of wetted charcoal, and this maintained in a large Petri dish at 25°C for six days. These faecal cultures were performed to confirm that these primary infections occurred. On days 15 and 16 p.i. all rats were administered thiabendazole, as described by Paterson and Viney (2002), to remove the worm infections. We confirmed that this treatment was effective by faecal culture of treated animals.

Thirty two days later (*i.e.*, day 48 p.i.) the secondary infection was given (Table 1), which is day 0 post secondary infection (p.s.i.). Faecal samples were collected on days 6, 9, 13, 16 and 20 p.s.i., the faeces cultured as previously described (Viney, 1996) at 19°C for three days, and the number of larvae that developed in these cultures was used as a measure of the total viable egg output of the infection (Gemmill *et al.*, 1997). Two animals from each treatment group were killed on days 7 and 21 p.s.i. each, the small intestine removed and stored at −20°C for subsequent determination of the number of *S. ratti* parasitic females, as previously described (Wilkes *et al.*, 2004). Survivorship was calculated as the number of parasitic females in the rat divided by the secondary dose. The *per capita* fecundity was calculated as the number of larvae that developed in faecal cultures divided by the number of parasitic females in the rat (Paterson and Viney, 2002).

Previous work has shown that in the presence of a host immune response, *S. ratti* parasitic females become positioned more posteriorly in the host small intestine (Kimura, *et al.*, 1999; Wilkes *et al.*, 2004). In this work, we have used part of the small intestine for immunological analyses (below). Therefore we will have underestimated the number of parasitic females (*i.e.* survivorship) and, consequently, overestimated *per capita* fecundity (Bleay *et al.*, 2007). It is not known how the different primary infection treatments (Table 1) may affect the intestinal position of *S. ratti*. Therefore the possibility exists that these different treatments may affect differently the underestimate of survivorship and the overestimate of *per capita* fecundity.

For all treatments, the concentration of *S. ratti*-specific immunoglobulin G_1 (IgG_1), IgG_{2a} and IgG_{2b} and total IgE all in serum and *S. ratti*-specific IgA and rat mast cell protease II both in intestinal tissue, was determined for animals sacrificed at days 7 and 21 p.s.i., all as previously described (Wilkes *et al.*, 2007). The intestinal tissue used for immunological analyses was 5cm of gut distal to the first 10% by length of the small intestine. Thus, the measures of the number of parasitic females in the gut, excludes any that were in this region (above). For two animals additional tissue was taken: for one rat with the PBS control primary - 30 dose secondary sacrificed on day 21 p.s.i., 12cm of gut was used and, for one rat with the treatment *S. ratti* 750 dose primary - 750 dose secondary sacrificed on day 21 p.s.i., 6 cm was used.

In addition, for the same animals in the *S. ratti* primary - *S. ratti* secondary and the PBS control primary - *S. ratti* secondary treatments (Table 1) the concentration of the following cytokines were measured: interleukin 4 (IL4), interleukin 13 (IL13), and interferon γ (IFNγ) from both spleen and mesenteric lymph node (MLN) cells stimulated with *S. ratti* parasitic female antigen all as previously described (Wilkes *et al.*, 2007). Spleens and MLNs were collected from animals sacrificed at days 7 and 21 p.s.i.

Europe PMC Funders Author Manuscripts

Statistical analysis

All analyses were conducted in R v2.7.0 (www.r-project.org). Analyses of *S. ratti* survivorship and *per capita* fecundity were performed using a generalised linear model (GLM) with a negative binomial error distribution (using the parameterization described in Wilson and Grenfell (1997)) and followed that described previously (Paterson and Viney, 2002). For survivorship, the dependent variable was the number of parasitic females. In order to express survivorship as the proportion of parasitic females in a host relative to the dose of iL3s administered, the dose of iL3s administered was used as an offset variable (*i.e.* a parameter value specified *a priori* rather than estimated from the data) (Crawley, 2002; Paterson and Viney, 2002). For *per capita* fecundity, the number of larvae developing in faecal cultures was the dependent variable and the number of parasitic females was used as an offset variable. Analyses of survivorship and *per capita* fecundity excluded those animals receiving PBS controls in the secondary infection (Table 1).

Deletion testing was used to derive minimal models for survivorship and *per capita* fecundity; *i.e.* models that contained only significant terms and for which no further significant terms could be added (Crawley, 2002). Likelihood ratio (LR) tests were used to assess significance of terms. All terms were fitted as factors (*i.e.* discrete variables). For both survivorship and *per capita* fecundity, these minimal models were derived by successive deletion of terms from a maximal model that consisted of BLOCK as a main effect (two level factor: replicates 1 and 2) and of the main effects of, and second order interactions between, SECONDARY DOSE (two level factor: 30 vs. 750 *S. ratti* iL3s), PRIMARY DOSE (7 level factor: PBS vs. 30 *N. brasiliensis* iL3s vs. 750 *N. brasiliensis* iL3s vs. 30 *S. venezuelensis* iL3s vs. 750 *S. venezuelensis* iL3s vs. 30 *S. ratti* iL3s vs. 750 *S. ratti* iL3s) and TIME (two level factor: days 7 vs. 21 p.s.i.). Thus the formula for the terms to be tested in the maximal model was: BLOCK + (PRIMARY DOSE + SECONDARY DOSE + TIME)^2.

Deletion testing was also used to determine the significance of factor levels. Thus, to determine whether the primary dose affected either survivorship or *per capita* fecundity, animals that received either 30 or 750 iL3s as a secondary dose were grouped together within each species to give a four level factor of primary infection that specified only the species, not the number, of iL3s (PRIMARY SPP.: PBS vs. *N. brasiliensis* vs. S. *venezuelensis* vs. *S. ratti*). LR tests were then performed between models containing either the 7 level PRIMARY DOSE factor or the four level PRIMARY SPP. factor to determine the significance of deleting factor levels associated with number of iL3s administered in the primary dose. Similarly, to determine whether a hetero-specific primary infection affected either *S. ratti* survivorship or *per capita* fecundity (*i.e.* whether primary infection with *N. brasiliensis* or *S. venezuelensis* was equivalent to a sham primary infection with PBS), animals receiving either PBS, *N. brasiliensis* or *S. venezuelensis* in a primary infection were combined into a single group and LR tests performed to determine the significance of factor levels for *N. brasiliensis* and *S. venezuelensis*.

Analyses of immune parameters included all animals (*i.e.* including those of secondary infection dose 0 (Table 1)) and followed the methods described in Paterson *et al.* (2008). Briefly, immune parameters were, where possible, normalised by a Box-Cox transformation $\left(y' = \frac{y^{\lambda} - 1}{\lambda}\right)$ identified by maximum likelihood such that the residuals from a linear model of (TIME X SECONDARY DOSE + PRIMARY DOSE) conformed as closely as possible to a normal distribution. Values of λ found were −0.4 for IgE, −0.18 for IgG_1, −1.01 for RMCPII, 0.02 for IL4 MLN, 0.02 for IFNγ MLN, −0.1 for IFNγ Spleen, 0.1 for IL13 MLN and 0.26 for IL13 Spleen and these were then analysed using linear models. No satisfactory transformations for IgA, IgG_{2a}, IgG_{2b} or IL4 Spleen were found and so these were analysed using GLMs with presence/absence of a detectable level of immunoglobulin isotype or cytokine (relative to corresponding negative controls). Significance of terms was determined using deletion testing from maximal models as for survivorship and *per capita* fecundity (as above) with the exception that secondary dose was fitted as a three level factor (0 vs. 30 vs. 750 iL3s) and that the significance of factor levels within the secondary infection were tested. Thus, animals that received either 30 or 750 *S. ratti* iL3s in a secondary infection were grouped together to give a two level factor (0 vs. 30 or 750 iL3s) and compared against the three level factor (0 vs. 30 vs. 750 iL3s). F tests were used in the case of linear models and LR tests were used in the case of GLMs.

Results and Discussion

Effects of primary infections on the survivorship of S. ratti *secondary infections*

Fewer *S. ratti* parasitic females were present in hosts previously exposed to *S. ratti* and this effect was density-dependent; the greater the dose given in a primary infection the fewer parasitic females were observed in the secondary infection (PRIMARY DOSE, LR test = 168.1, d.f. = 1, $p < 0.001$) (Table 2 and Figure 1). Analogously, there was a density-dependent effect of the secondary infection to reduce the survivorship of the secondary infection (SECONDARY DOSE X TIME, LR test = 198.5, d.f. = 1, $p < 0.001$) (Table 2). These results are fully consistent with previous observations of these phenomena (Paterson and Viney, 2002; Bleay *et al.*, 2007). A hetero-specific primary infection of *N. brasiliensis* or of *S. venezuelensis* did not affect the *S. ratti* survivorship in the secondary infection. That is, there was no significant difference between control (PBS), *S. venezuelensis* or *N. brasiliensis* primary infections in the survivorship of a secondary *S. ratti* infection. Therefore, *S. ratti* survivorship is not affected by these hetero-specific prior infections.

Effects of primary infections on the per capita *fecundity of* S. ratti *secondary infections*

Per capita fecundity declined between days 7 and 21 p.s.i. in all groups, consistent with our previous findings (Paterson and Viney, 2002). There was no consistent effect of primary infection on the fecundity of secondary infections on both days 7 and 21 p.s.i. However, the *per capita* fecundity of *S. ratti* secondary infections on day 21 p.s.i. was different between the control, PBS treated group and the infected groups (TIME X PRIMARY SPP., LR test = 15.3, d.f. = 1, $p < 0.001$) (Table 2 and Figure 2). But, the direction of this effect is counterintuitive, because the *S. ratti* fecundity of the control, PBS treated group was lower than that of the primary infected groups (Figure 2). Among the primary infected groups, the reduction in fecundity between days 7 and 21 p.s.i. was approximately equivalent (and not statistically distinguishable) between the *N. brasiliensis*, S. *venezuelensis* and *S. ratti* infection groups (Figure 2); no effect of primary dose was observed. We note that accurate estimates of *per capita* fecundity are difficult for day 21 p.s.i., since approximately half of the animals no longer had worms and hence are excluded from the analysis; the remainder had very low numbers of parasitic females. Moreover, the sample size for control, PBS treated group on day 21 p.s.i. is four, which is half that of the other primary infection groups (because different primary dose groups (Table 1) are grouped together). Overall, our results clearly show that neither hetero- nor con-specific primary infection reduced the fecundity of subsequent *S. ratti* infection; the observed effect of the PBS treatment should be interpreted with caution.

Therefore, our hypothesis of the effect of prior infection on *S. ratti* components of fitness is only supported for con-specific infection. However, it has been previously observed that immunisation with 3,000 larvae of *N. brasiliensis* significantly affected the survivorship and fecundity of a secondary *S. ratti* infection (Nawa *et al.*, 1982). This therefore suggests that comparatively very high doses, can induce detectable hetero-specific effects.

Associations between immune parameters and the survivorship and per capita *fecundity of secondary infections*

We extended this analysis (above) to determine whether there were any additional statistically detectable effects of measures of the host immune response on the survivorship and fecundity of secondary *S. ratti* infections. To do this, each of the immune parameters were added to these statistical models (Table 2) either as main effects or as interactions with time. Data for the concentration of immunglobulins and RMCPII were available for all infection groups (Table 1); data for the concentration of cytokines were only available for *S. ratti* or PBS control primary infections (Table 1).

S. ratti survivorship was negatively associated with the concentration of IL13 produced by MLN cells (IL13 MLN, LR test = 56.0, d.f. = 1, $p < 0.001$) (Table 3). This occurred as a main effect, such that there was no difference in the effect between days 7 and 21 p.s.i. IL13 has previously been identified to be important in protective immune responses in helminth infections (Finkelman *et al.*, 1999).

S. ratti per capita fecundity was associated with the concentration of *S. ratti*-specific IgG_1 and the concentration of IL4 and IL13 produced by MLN cells, as interactions with time p.s.i. (TIME X IgG_1, LR test = 4.9, d.f. = 1, $p < 0.05$; TIME X IL4 MLN, LR test = 19.1, d.f. = 1, $p < 0.001$; TIME X IL13 MLN, LR test = 16.2, d.f. = 1, $p < 0.001$) (Table 4). These effects occurred such that IgG_1, IL4 and IL13 concentrations were negatively associated with fecundity on day 7 p.s.i.

Previously, we found that *S. ratti* survivorship was negatively related to the concentration of parasite-specific IgG_1, IgA and IL4 MLN, whereas fecundity was negatively related to the concentration of IgA only (Bleay *et al.*, 2007). Therefore there is some qualitative overlap between the immunological results of these two studies, in that the concentration of IL4 MLN and IgG_1 is associated with components of *S. ratti* fitness in both studies, though the details of these effects differ between the studies. Note, the prior study did not analyse IL13. It is notable that here we have not detected any effect of the concentration of IgA, in contrast to the previous study (Bleay *et al.*, 2007). These apparently different observations may be due to temporal effects. Previously it was observed that the concentration of IgA changed with time (Bleay *et al.*, 2007). Here, IgA concentration was only measured at two points, and this may not have detected such temporal changes in its concentration.

Effects of primary and secondary infections on immune parameters

A secondary infection with *S. ratti* resulted in a significantly greater concentration of the following measured immune parameters, compared with a control PBS secondary infection treatment: IL4, IL13 and IFNγ all produced by MLN cells, IL13 produced by spleen cells, *S. ratti*-specific IgG_1, total IgE and RMCPII (Table 5). The concentration of IL4 produced by MLN cells was positively affected by the dose of the secondary infection (SECONDARY DOSE, $F_{2,32} = 14.29$, $p < 0.001$) (Table 5). This has been previously observed in *S. ratti* primary infections (Bleay *et al.*, 2007). Further, as shown in Figure 3, the concentration of IgG_1 was also affected by the dose of the secondary infection at 21 days p.s.i., but not at 7 days p.s.i. (TIME X SECONDARY DOSE, $F_{2,73} = 18.81$, $p < 0.001$) (Table 5). The effect of IL13 concentration produced by both MLN and spleen cells are consistent with the observed association between the concentration of this cytokine and survivorship.

A primary infection significantly affected the concentration of the following measured immune parameters in a secondary *S. ratti* infection: IL4 and IFNγ produced by MLN cells, *S. ratti*-specific IgG_1, total IgE and RMCPII. A primary *S. ratti* infection, regardless of the dose, resulted in a higher concentration of IL4 and lower concentration of IFNγ by MLN cells (IL4 MLN, PRIMARY SPP. *S. RATTI*, $F_{1,32} = 6.38$, $p < 0.05$; IFNγ MLN, PRIMARY SPP. *S. RATTI*, $F_{1,32}$ =

Europe PMC Funders Author Manuscripts

5.21, $p < 0.05$) (Table 5). Thus, prior history of exposure to *S. ratti* suppresses inflammatory Th1-type responses (*i.e.* IFNγ) and further promotes Th2-type responses (*i.e.* IL4) in an *S. ratti* secondary infection, consistent with the Th2-bias associated with protective, acquired immune responses to nematode infections (Bancroft, 1994; Turner, 2003) Further, there was a positive *S. ratti* primary dose-dependent effect on the *S. ratti*-specific IgG_1 concentration in the secondary infection (TIME X PRIMARY SPP. *S. RATTI*, $F_{2,73} = 4.13$, $p < 0.05$). That is, a *S. ratti* primary infection of 750 iL3s resulted in a greater concentration of IgG_1 in a secondary infection, compared with primary dose of 30 iL3s. This effect occurred on both days 7 and 21 p.s.i., but was comparatively somewhat stronger on day 7 p.s.i. (Figure 3). These results suggest that IgG_1 has a central role in the host immune response to *S. ratti*, since it is affected in a density-dependent manner by both prior and current infections. These results are therefore consistent with our hypothesis, namely that host prior infection can enhance anti-*S. ratti* immune responses.

Hetero-specific primary infections only affected the concentration of total IgE and RMCPII. A primary infection of either *N. brasiliensis* or *S. venezuelensis* increased the concentration of IgE, compared with control (PBS) treated animals, during the secondary infection (PRIMARY SPP., $F_{4,78}= 2.89$, $p <0.05$) (Table 5). Both *N. brasiliensis* and *S. venezuelensis* had effects of the same magnitude on the concentration of IgE; these effects were, in turn, less than those caused by a primary *S. ratti* infection (Table 5). There was no effect of the dose of these hetero-specific primary infections on the concentration of IgE during the secondary infection. However, there was a positive effect of the dose of the primary *S. ratti* infection on the concentration of IgE during the secondary infection (Table 5). Primary infections with *N. brasiliensis, S. venezuelensis* or *S. ratti* increased the concentration of RMCPII compared with control, PBS treated animals, on day 7 p.s.i., but not on day 21 p.s.i. (TIME X PRIMARY SPP.,$F_{3,74} = 2.81$, $p < 0.05$, Table 5). There was no difference between the effect of the primary infecting species (*N. brasiliensis, S. venezuelensis* or *S. ratti*) on the concentration of RMCPII during the secondary infection (Figure 4).

Both the concentration of total IgE and RMCPII are measures of non-specific effectors of the host immune response elicited by gastrointestinal nematode infection. Therefore these hetero-specific prior infections resulted in some enhancement of the anti-*S. ratti* immune response, though there was no difference between *S. venezuelensis* and *N. brasiliensis* prior infection, nor was there an effect of their dose. These results therefore support, in-part, our hypothesis of the effect of host prior infection on anti-*S. ratti* immune responses.

In conclusion, this experiment has shown that the strongest effect on *S. ratti* is the effect of a con-specific prior infection on *S. ratti* survivorship. We have also found that a host con- or hetero-specific prior infection can enhance the host immune response against a secondary *S. ratti* infection, but in different ways. Thus, there is a primary con-specific, dose-dependent effect on the concentration of IgG_1, an isotype previously identified to be important in *S. ratti* infections (Wilkes *et al.*, 20007; Bleay *et al.*, 2007). In contrast there are dose-independent non-specific immune effects (IgE and RMCPII) of hetero-specific host prior infection. These results therefore suggest that the principal immunological effect against a nematode infection is elicited by that infection itself and by its dose, that prior infections have smaller, mainly dose-independent, effects and hetero-specific infections have the least effect.

Acknowledgments

We would like to thank Maayan Navellou for technical help. This work was funded by a grant from the Wellcome Trust.

Europe PMC Funders Author Manuscripts

References

Bancroft AJ, Else KJ, Grencis RK. Low-level infection with *Trichuris muris* significantly affects the polarization of the CD4 response. European Journal of Immunology. 1994; 24:3113–3118. [PubMed: 7805740]

Behnke JM. Structure in parasite component communities in wild rodents: predictability, stability, associations and interactions .... or pure randomness? Parasitology. 2008; 135:751–766. [PubMed: 18371244]

Bleay C, Wilkes CP, Paterson S, Viney ME. Density-dependent immune responses against the gastrointestinal nematode *Strongyloides ratti*. International Journal for Parasitology. 2007; 37:1501–1509. [PubMed: 17597133]

Crawley, MJ. Statistical computing: an introduction to data analysis using S-plus. Wiley; Chichester: 2002.

Finkelman FD, Shea-Donohue T, Goldhill J, Sullivan CA, Morris SC, Madden KB, Gause WC, Urban JFJ. Cytokine regulation of host defense against parasitic gastrointestinal nematodes: Lessons from studies with rodent models. Annual Review of Immunology. 1997; 15:505–533.

Finkelman FD, Wynn TA, Donaldson DD, Urban JFJ. The role of IL-13 in helminth-induced inflammation and protective immunity against nematode infections. Current Opinion in Immunology. 1999; 11:420–426. [PubMed: 10448138]

Gemmill AW, Viney ME, Read AF. Host immune status determines sexuality in a parasitic nematode. Evolution. 1997; 51:393–401.

Harvey SC, Gemmill AW, Read AF, Viney ME. The control of morph development in the parasitic nematode *Strongyloides ratti*. Proceedings of the Royal Society of London Series B. 2000; 267:2057–2063. [PubMed: 11416909]

Keymer AE. Density-dependent mechanisms in the regulation of intestinal helminth populations. Parasitology. 1982; 84:573–587. [PubMed: 7099714]

Kimura E, Shintoku Y, Kadosaka T, Fujiwaram M, Kondom S, Itoh M. A second peak of egg excretion in *Strongyloides ratti*-infected rats: Its origin and biological meaning. Parasitology. 1999; 119:221–226. [PubMed: 10466130]

Moqbel R, Wakelin D. *Trichinella spiralis* and *Strongyloides ratti*: Immune interaction in adult rats. Experimental Parasitology. 1979; 47:65–72. [PubMed: 421767]

Nawa Y, Mimori T, Korenaga M, Tada I. Stage-specific cross-resistance between *Nippostrongylus brasiliensis* and *Strongyloides ratti* (Nematoda) in rats. Journal of Parasitology. 1982; 68:804–808. [PubMed: 7131185]

Paterson S, Viney ME. Host immune responses are necessary for density-dependence in nematode infections. Parasitology. 2002; 125:283–292. [PubMed: 12358425]

Paterson S, Wilkes CP, Bleay C, Viney ME. Immunological responses elicited by different infection regimes with *Strongyloides ratti*. PLoS One. 2008; 3:e2509. [PubMed: 18575588]

Turner JD, Faulkner H, Kamgno J, Cormont F, van Snick J, Else KJ, Grencis RK, Behnke JM, Boussinesq M, Bradley JE. Th2 cytokines are associated with reduced worm burdens in a human intestinal helminth infection. Journal of Infectious Diseases. 2003; 188:1768–1775. [PubMed: 14639550]

West SA, Gemmill AW, Graham A, Viney ME, Read AF. Immune stress and facultative sex in a parasitic nematode. Journal of Evolutionary Biology. 2001; 14:333–337.

Wilkes CP, Bleay C, Paterson S, Viney ME. The immune response during a *Strongyloides ratti* infection of rats. Parasite Immunology. 2007; 29:339–346. [PubMed: 17576363]

Wilkes CP, Thompson FJ, Gardner MP, Paterson S, Viney ME. The effect of the host immune response on the parasitic nematode *Strongyloides ratti*. Parasitology. 2004; 128:661–669. [PubMed: 15206469]

Wilson K, Grenfell BT. Generalized linear modelling for parasitologists. Parasitology Today. 1997; 13:33–38. [PubMed: 15275165]

Viney ME. Developmental switching in the parasitic nematode *Strongyloides ratti*. Proceedings of the Royal Society of London Series B. 1996; 263:201–208. [PubMed: 8728983]

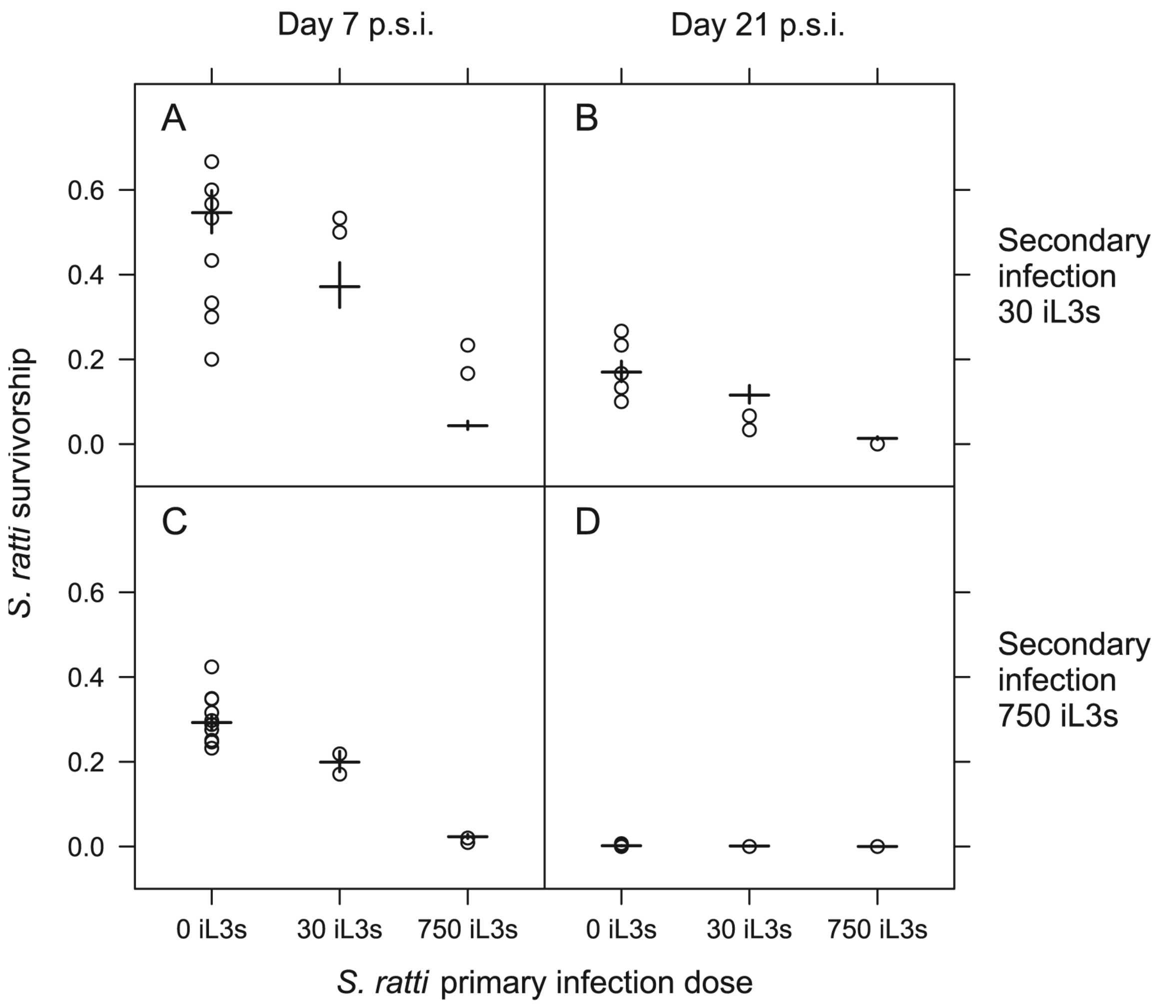

Figure 1.

The *S. ratti* survivorship for three primary *S. ratti* doses and secondary *S. ratti* infections at doses of 30 iL3s (A, B) and 750 iL3s (C, D) and at days 7 (A, C) and 21 p.s.i. (B, D). Results for individual animals are circles, with model estimates from Table 2 shown as horizontal bars with standard errors on these estimates indicated by vertical bars. Animals receiving *N. brasiliensis* or *S. venezuelensis* primary infections are included in the '0 iL3s' group since these hetero-specific infections had no detectable effect on subsequent *S. ratti* survivorship.

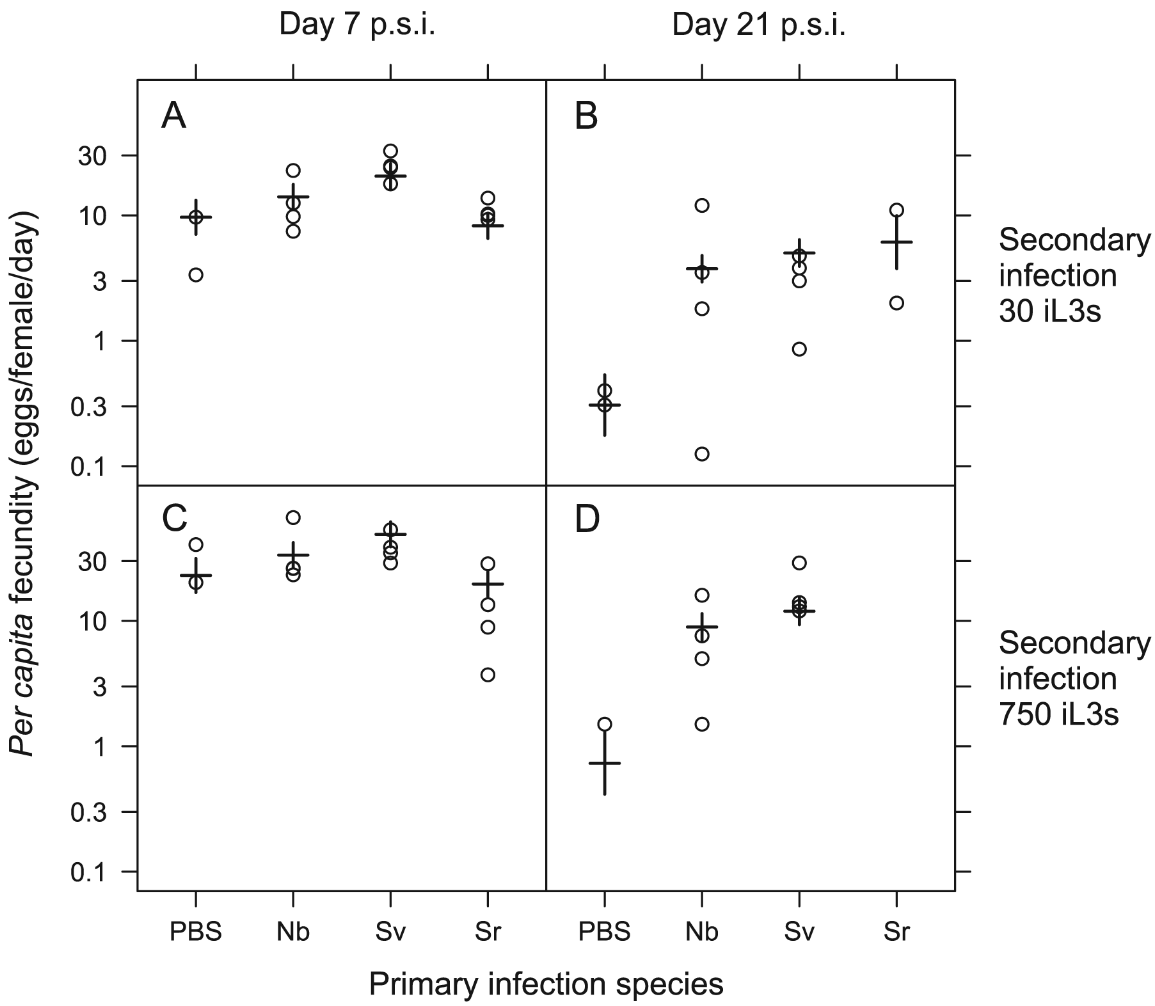

Figure 2.

The *S. ratti per capita* fecundity for con- or hetero-specific primary infections and secondary *S. ratti* infections at doses of 30 iL3s (A, B) and 750 iL3s (C, D) and at days 7 (A, C) and 21 p.s.i. (B, D). Results for individual animals are circles, with model estimates from Table 2 shown as horizontal bars with standard errors on these estimates indicated by vertical bars. Data are plotted on a log scale. The primary infections were: PBS controls (PBS), *N. brasiliensis* (Nb), *S. venezuelensis* (Sv) and *S. ratti* (Sr). The different doses delivered in the primary infection (Table 1) for each species are grouped together since the dose of the primary infection had no detectable effect on the *per capita* fecundity of *S. ratti* secondary infections.

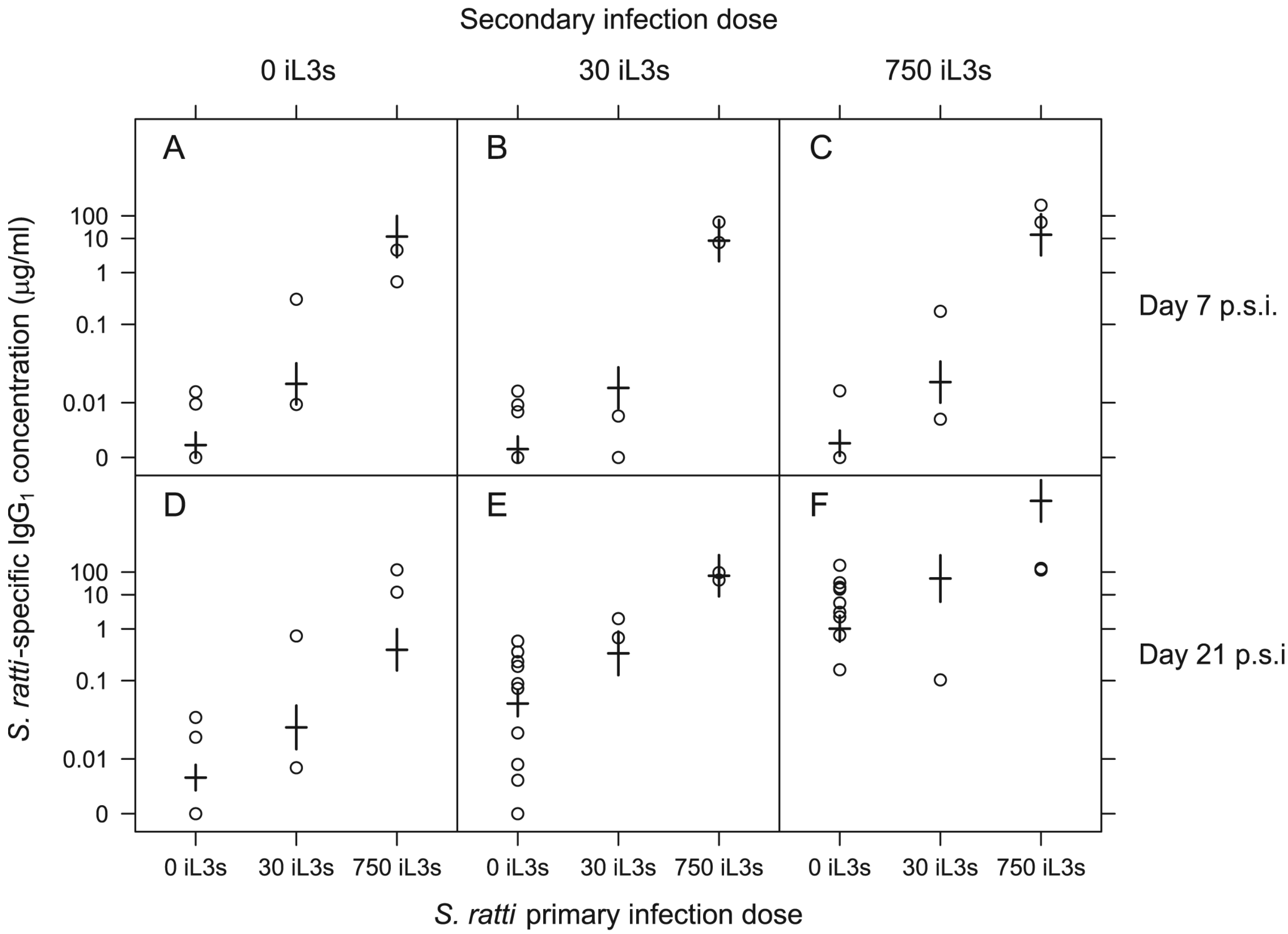

Figure 3.

Anti-*S. ratti* IgG_1 concentrations for three primary *S. ratti* doses and secondary *S. ratti* infections at doses of 0 (A, D), 30 (B, E) and 750 iL3s (C, F) at days 7 (A, B, C) and 21 p.s.i. (D, E, F). Results for individual animals are circles, with model estimates from Table 5 shown as horizontal bars with standard errors on these estimates indicated by vertical bars. Data are plotted on a Box-Cox transformed scale. Animals receiving *N. brasiliensis* or *S. venezuelensis* primary infections are included in the '0 iL3s' group since these hetero-specific infections had no detectable effect on subsequent anti-*S. ratti* IgG_1 concentration.

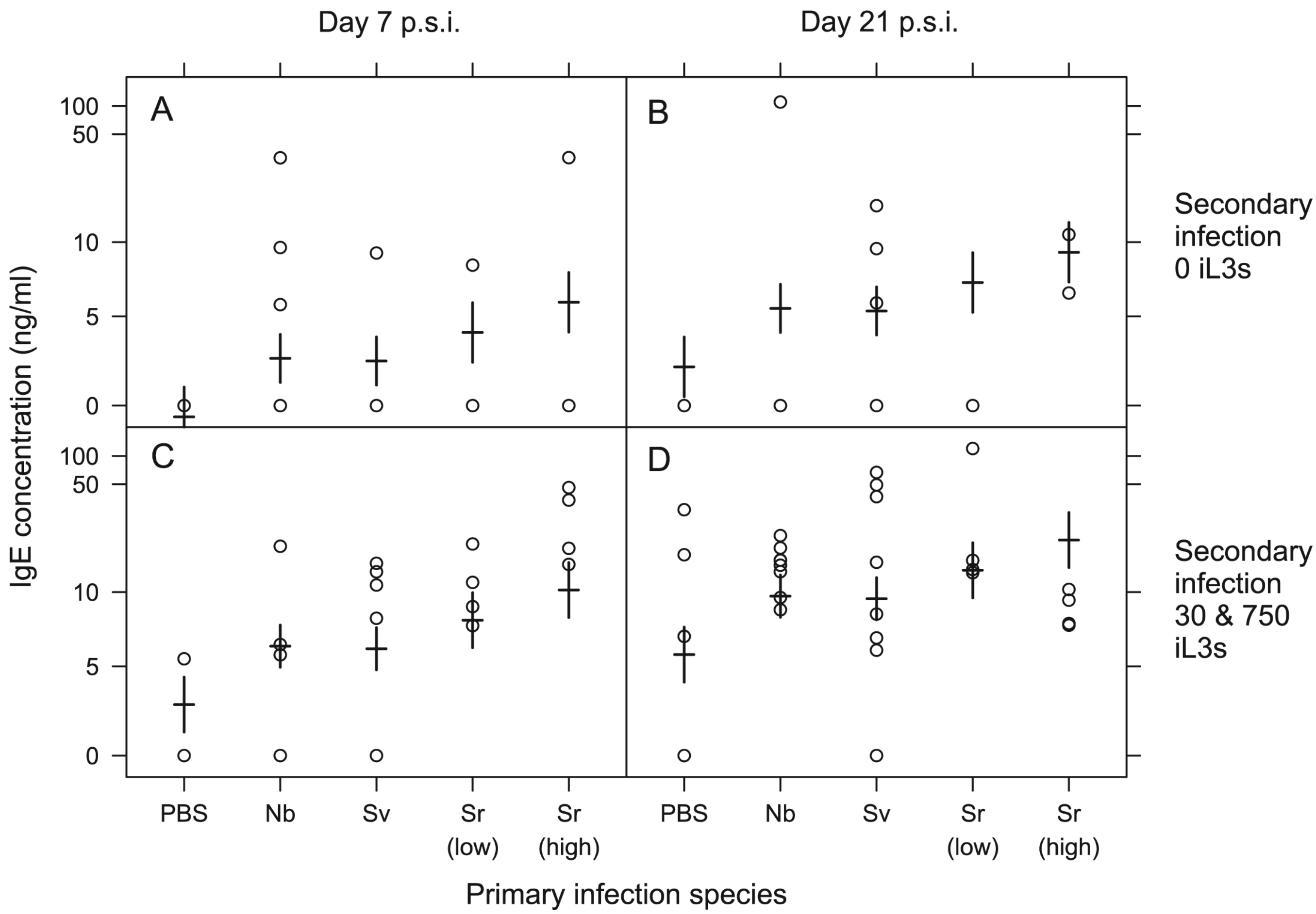

Figure 4.

Total IgE concentrations for con- or hetero-specific primary infections and secondary *S. ratti* infections having received either a dose 0 iL3s (A, B) or a dose of either 30 or 750 iL3s (C, D) at days 7 (A, C) and 21 p.s.i. (B, D). Results for individual animals are circles, with model estimates from Table 5 shown as horizontal bars with standard errors on these estimates indicated by vertical bars. Data are plotted on a Box-Cox transformed scale. The primary infections were: PBS controls (PBS), *N. brasiliensis* (Nb), *S. venezuelensis* (Sv), S. ratti 30 iL3s (Sr low) and *S. ratti* 750 iL3s (Sr high). The different doses delivered in the primary infection for *N. brasiliensis* and, *S. venezuelensis* are grouped together since the doses of these primary infections were not found to significantly affect IgE concentration during *S. ratti* secondary infections. Animals receiving 30 or 750 iL3s in a secondary infection were grouped together since there was no detectable difference between these doses with respect to their effect on IgE concentration.

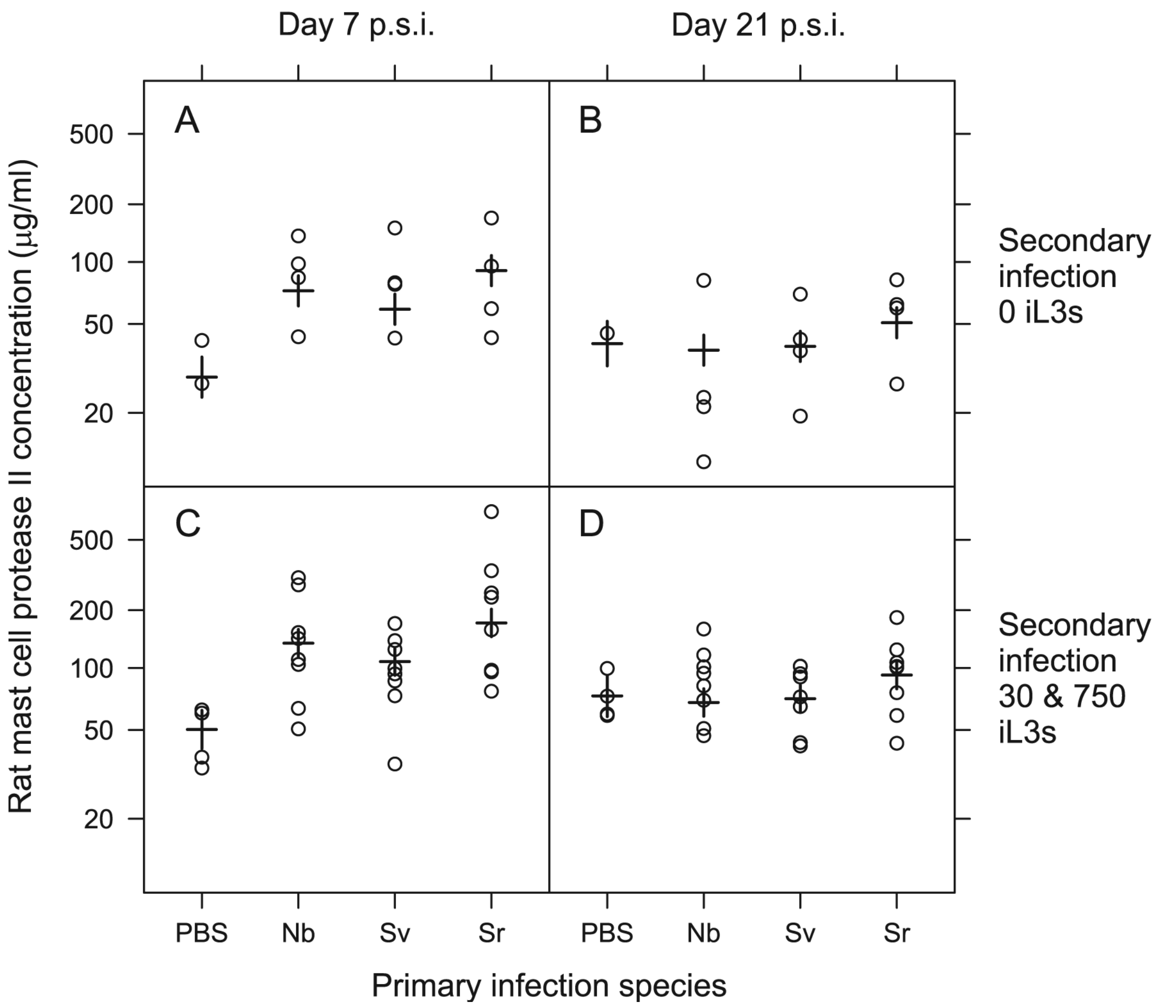

Figure 5.

RMCPII concentrations for con- or hetero-specific primary infections and secondary *S. ratti* infections having received either a dose 0 iL3s (A, B) or either 30 or 750 iL3s (C, D) at days 7 (A, C) and 21 p.s.i. (B, D). Results for individual animals are circles, with model estimates from Table 5 shown as horizontal bars with standard errors on these estimates indicated by vertical bars. Data are plotted on a Box-Cox transformed scale. The primary infections were: PBS controls (PBS), *N. brasiliensis* (Nb), *S. venezuelensis* (Sv) and *S. ratti* (Sr). The different doses delivered in the primary infection for each species are grouped together since the dose of the primary infection was not found to significantly affect RMCPII concentration during *S. ratti* secondary infections. Animals receiving 30 or 750 iL3s in a secondary infection were grouped together since there was no detectable difference between these doses with respect to their effect on RMCPII concentration.

Table 1

Experimental design showing the 21 treatments. Immunology: 1 indicates that immunoglobulin and RMCP concentrations were measured, 2 indicates that cytokine concentrations were additionally measured, all as detailed above

Primary infection		**Secondary infection**		
Species	**Dose**	**Species**	**Dose**	**Immunology**
S. ratti	30	*S. ratti*	0	2
S. ratti	30	*S. ratti*	30	2
S. ratti	30	*S. ratti*	750	2
S. ratti	750	*S. ratti*	0	2
S. ratti	750	*S. ratti*	30	2
S. ratti	750	*S. ratti*	750	2
S. venezuelensis	30	*S. ratti*	0	1
S. venezuelensis	30	*S. ratti*	30	1
S. venezuelensis	30	*S. ratti*	750	1
S. venezuelensis	750	*S. ratti*	0	1
S. venezuelensis	750	*S. ratti*	30	1
S. venezuelensis	750	*S. ratti*	750	1
N. brasiliensis	30	*S. ratti*	0	1
N. brasiliensis	30	*S. ratti*	30	1
N. brasiliensis	30	*S. ratti*	750	1
N. brasiliensis	750	*S. ratti*	0	1
N. brasiliensis	750	*S. ratti*	30	1
N. brasiliensis	750	*S. ratti*	750	1
Control (PBS)	0	*S. ratti*	0	2
Control (PBS)	0	*S. ratti*	30	2
Control (PBS)	0	*S. ratti*	750	2

Table 2

The effects of primary infections on *S. ratti* survivorship and *per capita* fecundity

	Term	Coefficient	LR test[a]	P value[a]
Survivorship [b]	Intercept	−0.605 ± 0.094	-	
	Secondary dose (750 iL3s)	−1.17 ± 0.17	34.38	<0.001
	Time (Day 21 p.s.i.)	−0.624 ± 0.106	55.13	<0.001
	Primary dose (30 *S. ratti* iL3s)[c]	−0.385 ± 0.126		
	Primary dose (750 *S. ratti* iL3s)[c]	−2.54 ± 0.21	168.13	<0.001
	Secondary dose × Time	−3.96 ± 0.32	198.50	<0.001
Fecundity [d]	Intercept	2.27 ± 0.31		
	Secondary dose (750 iL3s)	0.87 ± 0.18	21.01	<0.001
	Time (Day 21 p.s.i.)	−3.45 ± 0.63	39.40	<0.001
	Primary spp. (*N. brasiliensis*)	0.375 ± 0.366		
	Primary spp. (*S. venezuelensis*)	0.755 ± 0.366		
	Primary spp. (*S. ratti*)	−0.157 ± 0.367	13.18	<0.01
	Time × Primary spp. (*N. brasiliensis*)	2.13 ± 0.70		
	Time × Primary spp. (*S. venezuelensis*)	2.04 ± 0.70		
	Time × Primary spp. (*S. ratti*)	3.15 ± 0.82	15.31	<0.01

[a]Likelihood ratio test presented for deletion of individual terms. For factors, tests refer to simultaneous deletion of all factor levels.

[b]2 × log-likelihood = −318.31 with 50 residual degrees of freedom, overdispersion parameter $k = 29.6 \pm 14.5$.

[c]Factor levels for primary dose were grouped such that animals receiving either PBS, *N. brasiliensis* or *S. venezuelensis* in a primary infection were compared with those receiving 30 and 750 *S. ratti* iL3s as a primary infection.

[d]2 × log-likelihood = −560.87 with 40 residual degrees of freedom, overdispersion parameter $k = 2.83 \pm 0.63$.

Table 3

The association between *S. ratti* survivorship and IL13 produced by mesenteric lymph node cells

Term	Coefficient	LR test[a]	P value[a]
Intercept	0.418 ± 0.229		
Time (Day 21 p.s.i.)	−2.23 ± 0.32	49.37	<0.001
Secondary dose (750 iL3s)	−0.828 ± 0.209	28.98	<0.001
Primary spp. *S. ratti* (30 iL3s)	−0.797 ± 0.260	-	-
Primary spp. *S. ratti* (750 iL3s)	−2.31 ± 0.20	45.49	<0.001
Time × Secondary dose (750 iL3s)	−4.16 ± 0.78	49.85	<0.001
IL13 MLN	−0.646 ± 0.088	56.01	<0.001

2 × log-likelihood = −75.20 with 13 residual degrees of freedom, overdispersion parameter $k > 100$

[a]Likelihood ratio test presented for deletion of individual terms. For factors, tests refer to simultaneous deletion of all factor levels.

Europe PMC Funders Author Manuscripts

Table 4

The association between *S. ratti* fecundity and immune parameters

	Term	Coefficient	LR test[a]	P value[a]
IgG_1 [b]	Intercept	2.06 ± 0.35		
	Time (Day 21 p.s.i.)	−3.48 ± 0.67	11.67	<0.001
	Secondary dose (750 iL3s)	0.723 ± 0.195	12.87	<0.001
	Primary spp. (*N. brasiliensis*)	0.390 ± 0.346	-	
	Primary spp. (*S. venezuelensis*)	0.759 ± 0.346	-	
	Primary spp. (*S. ratti*)	0.241 ± 0.444	12.12	<0.01
	Time × Primary spp. (*N. brasiliensis*)	2.09 ± 0.68	-	
	Time × Primary spp. (*S. venezuelensis*)	2.19 ± 0.69	-	
	Time × Primary spp. (*S. ratti*)	2.64 ± 0.85	12.64	<0.01
	IgG_1	−0.320 ± 0.184	0.23	0.63
	Time × IgG_1	0.686 ± 0.297	4.99	<0.05
IL4 [c]	Intercept	1.28 ± 0.42		
	Time (Day 21 p.s.i.)	−3.26 ± 0.73	7.40	<0.01
	Secondary dose (750 iL3s)	0.988 ± 0.261	14.66	<0.001
	Primary spp. (*S. ratti*)	1.57 ± 0.63	1.08	0.30
	Time × Primary spp. (*S. ratti*)	2.13 ± 0.94	5.50	<0.05
	IL4 MLN	−1.30 ± 0.43	1.015	0.31
	Time × IL4 MLN	2.23 ± 0.54	19.17	<0.001
IL13 [d]	Intercept	3.16 ± 0.23		
	Time (Day 21 p.s.i.)	−5.02 ± 0.62	7.40	<0.01
	Secondary dose (750 iL3s)	0.724 ± 0.163	19.10	<0.001
	Primary spp. (*S. ratti*)	−0.510 ± 0.170	1.08	0.30
	Time × Primary spp. (*S. ratti*)	3.46 ± 0.58	42.55	<0.001
	IL13 MLN	−0.479 ± 0.152	0.41	0.52
	Time × IL13 MLN	2.07 ± 0.53	16.25	<0.001

[a] Likelihood ratio test presented for deletion of individual terms. For factors, tests refer to simultaneous deletion of all factor levels.

[b] 2 × log-likelihood = −555.89 with 38 residual degrees of freedom, overdispersion parameter $k = 3.18 \pm 0.73$

[c] 2 × log-likelihood = −168.595 with 10 residual degrees of freedom, overdispersion parameter $k = 3.18 \pm 0.73$

[d] 2 × log-likelihood = −106.54 with 6 residual degrees of freedom, overdispersion parameter $k = 33.1 \pm 18.9$

Europe PMC Funders Author Manuscripts

Table 5

The effects of primary and secondary infections on immune parameters

	Term	Coefficient	t[a]	P value[a]
IgE [b]	Intercept	−1.31 ± 0.31	−4.28	<0.001
	Time (Day 21 p.s.i.)	0.513 ± 0.194	2.65	<0.01
	Secondary dose (30 or 750 iL3s)[c]	0.639 ± 0.206	3.11	<0.01
	Primary spp. (*N. brasiliensis*)	0.600 ± 0.314	1.91	0.06
	Primary spp. (*S. venezuelensis*)	0.574 ± 0.314	1.83	0.07
	Primary spp. (*S. ratti*, 30 iL3s)	0.866 ± 0.363	2.39	<0.05
	Primary spp. (*S. ratti*, 750 iL3s)	1.18 ± 0.36	3.24	<0.01
IgG_1 [d]	Intercept	−0.830 ± 0.133	−6.24	-
	Time (Day 21 p.s.i.)	0.250 ± 0.189	1.32	0.19
	Secondary dose (30 iL3s)	−0.043 ± 0.177	−0.24	0.81
	Secondary dose (750 iL3s)	0.019 ± 0.177	0.11	0.92
	Primary spp. *S. ratti* (30 iL3s)	0.644 ± 0.209	3.08	<0.01
	Primary spp. *S. ratti* (750 iL3s)	2.19 ± 0.21	10.5	<0.001
	Time × Secondary dose (30 iL3s)	0.822 ± 0.250	3.29	<0.01
	Time × Secondary dose (750 iL3s)	1.55 ± 0.25	6.13	<0.001
	Time × Primary spp. *S. ratti* (30 iL3s)	−0.116 ± 0.309	−0.38	0.71
	Time × Primary spp. *S. ratti* (750 iL3s)	−0.849 ± 0.296	−2.87	<0.01
RMCPII [e]	Intercept	−1.55 ± 0.34	−4.58	-
	Secondary dose (30 or 750 iL3s)[c]	0.902 ± 0.182	4.97	<0.001
	Time (Day 21 p.s.i.)	0.564 ± 0.469	1.2	0.23
	Primary spp. (*N. brasiliensis*)	1.45 ± 0.39	3.75	<0.001
	Primary spp. (*S. venezuelensis*)	1.14 ± 0.40	2.95	<0.01
	Primary spp. (*S. ratti*)	1.79 ± 0.39	4.63	<0.001
	Time × Primary spp. (*N. brasiliensis*)	−1.56 ± 0.56	−2.76	<0.01
	Time × Primary spp. (*S. venezuelensis*)	−1.19 ± 0.56	−2.1	<0.05
	Time × Primary spp. (*S. ratti*)	−1.44 ± 0.56	−2.55	<0.05
IL4 MLN [f]	Intercept	−1.27 ± 0.27	−4.73	-
	Secondary dose (30 iL3s)	0.969 ± 0.295	3.28	<0.01
	Secondary dose (750 iL3s)	1.56 ± 0.30	5.30	<0.001
	Primary spp. *S. ratti* (30 or 750 iL3s)[g]	0.646 ± 0.256	2.53	<0.05
IL13 MLN [h]	Intercept	−0.315 ± 0.242	−1.30	-
	Time (Day 21 p.s.i.)	−0.856 ± 0.255	−3.36	<0.01
	Secondary dose (30 or 750 iL3s)[c]	1.27 ± 0.26	4.88	<0.001
IL13 spleen [i]	Intercept	−0.97 ± 0.21	−4.63	-
	Secondary dose (30 or 750 iL3s)[c]	1.46 ± 0.26	5.67	<0.001
IFNγ MLN[j]	Intercept	−0.529 ± 0.318	−1.67	-
	Time (Day 21 p.s.i.)	0.983 ± 0.259	3.79	<0.001
	Primary spp. *S. ratti* (30 or 750 iL3s)[g]	−0.628 ± 0.275	−2.28	<0.05

Term	Coefficient	t[a]	P value[a]
Secondary dose (30 or 750 iL3s)[c]	0.685 ± 0.275	2.49	<0.05

[a]Significance of individual terms are presented as estimates against a t distribution. F tests from deletion tests are presented in the text.

[b]2 × log-likelihood = −211.92 with 78 residual degrees of freedom

[c]Factor levels for secondary dose were grouped such that animals receiving a dose of 0 were compared with those receiving either 30 or 750 *S. ratti* iL3s.

[d]2 × log-likelihood = −98.70 with 73 residual degrees of freedom

[e]2 × log-likelihood = −183.40 with 74 residual degrees of freedom

[f]2 × log-likelihood = −74.56 with 32 residual degrees of freedom

[g]Factor levels for primary dose were grouped such that animals receiving either PBS, *N. brasiliensis* or *S. venezuelensis* were compared with those receiving 30 or 750 *S. ratti* iL3s as a primary infection.

[h]2 × log-likelihood = −65.88 with 29 residual degrees of freedom

[i]2 × log-likelihood = −77.16 with 34 residual degrees of freedom

[j]2 × log-likelihood = −79.89 with 32 residual degrees of freedom

Europe PMC Funders Author Manuscripts