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**Co-infection impacts on resistance and tolerance to nematodes differ between different mice genotypes**

**Application**: The impact of co-infection on the resistance and tolerance to an intestinal nematode was affected by host’ genetics, with susceptible hosts benefiting the most.

**Introduction:** There are two mechanisms by which hosts can defend against pathogen infection: resistance, the ability to clear pathogen load and tolerance, the ability to withstand pathogen impact (Raberg et al., 2007). To date, the relationship between resistance and tolerance has mostly been studied in the context of a single infection (Athanasiadou et al., 2015), but dynamics may be different when hosts are exposed to more than one pathogen. The aim of our study was to characterise the variation of host resistance and tolerance to an intestinal nematode of mice *(Heligmosomoides polygyrus)* during co-infection with another intestinal pathogen, Theiler’s murine encephalomyelitis virus (TMEV). Host responses against *H. polygyrus* are known to be at least partly, genetically controlled(Oleszak et al., 2004; Maizels et al., 2012).

Our hypothesis was tested in genotypes that differed in the susceptibility to *H.polygyrus*; we expected that susceptible mice would suffer more from the co-infection compared to resistant mice.

**Material and methods:** Three strains of mice were used: SJL mice (resistant to *H. polygyrus*), BALB/c mice (intermediate susceptibility to *H. polygyrus)*, and C57BL/6 (susceptible to *H. polygyrus*). Mice from each strain were divided into four infection treatment: co-infection (**Co-inf**), *H. polygyrus*-only (**Par**), TMEV-only (**Vir**), and non-infected (**Sham**). Co-inf and Par mice were administered with 0.2 ml of 250 *H. polygyrus* 3rd stage infective larvae (L3) orally at day 0 (D0) whereas 0.2 ml of water was administered for Vir and Sham mice at D0. Co-inf and Vir mice received 0.2 ml of 10^6 PFU TMEV at D8 whereas Par and Sham mice received 0.2 ml of DMEM (Dulbecco s Modified Eagles Medium). Mice were euthanised at **14 dpi** and **42 dpi**; these two time points represent expected nematode establishment and nematode clearance periods respectively, in the resistant genotype. Average daily gain (ADG) and feed intake (FI) were analysed using linear mixed model with ADG or FI as the dependent variable, and mouse strain, infection treatment, week post infection (WPI), and all statistically significant interactions as fixed effects and cage as a random effect. To assess the effect of mice genetics and treatment on the resistance against *H. polygyrus*, as measured by EIC and worm burden, a linear mixed model was used. To assess the effect of mice genetics and treatment on the tolerance against *H. polygyrus*, a linear mixed model was used with carcass weight as dependant variable and parasite burden (total worms and total EIC) as independent variables. Mouse strains, infection treatment, and all statistically significant interactions as fixed effects, and individual mouse as random effect were used in both resistance and tolerance analysis. Statistical analysis was performed in R Studio

**Results:** In the susceptible to *H. polygyrus* C57BL/6 mice, ADG (0.08 g/day) and FI (0.07g/day) was higher in Co-inf compared to Par, Vir, and Sham at 2 weeks post infection (*P*<0.001). However, in the BALB/c mice, co-infection treatment reduced 20% of ADG compared to mice receiving *H. polygyrus*-only at 2 weeks post infection (*P*=0.034); 10% of ADG loss was observed in the resistant to *H. polygyrus* SJL mice (*P*<0.001). When compared to *H. polygyrus*-only C57BL/6 mice, co-infected C57BL/6 mice had 15% lower EIC (*P*<0.001), and 20% worm counts (*P*<0.05), whereas Co-inf BALB/c mice showed 5% elevated EIC (*P*<0.001) and 10% worm counts (*P*<0.05) compared to *H. polygyrus*-only mice. Co-inf did not have any impact on resistance traits in SJL mice. In the second time point tested, 25% reduction EIC (*P*<0.001) and 30% decrease in worm burden (*P*<0.001) were showed in co-inf C57BL/6 mice compared to *H. polygyrus* only infected counterparts. On the contrary, Co-inf BALB/c mice increased EIC and worm burden up to 25% compared to *H. polygyrus* only mice (*P*<0.001). Compared to *H. polygyrus* only SJL mice, Co-inf mice were 5% more tolerant (*P*<0.05). Co-inf C57BL/6 mice tended to be more tolerant (*P*=0.058) whereas Co-inf BALB/c mice tended to be less tolerant than their *H.polygyrus* only counterparts (*P*=0.051).

**Conclusion:** Our data showed that the impact of co-infection with two intestinal pathogens resulted in significant variation on host resistance and tolerance to *H.polygyrus* in three inbred mouse strains. Contrary to expectation, mice susceptible to *H.polygyrus* benefited most from co-infection, as their resistance was improved compared to *H.polygyrus-*only counterparts. On the other hand, mice resistant to *H.polygyrus* showed a benefit in their tolerance compared to *H.polygyrus* only mice. The underlying mechanisms of these co-infection phenotypes are currently investigated.

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