

The influence of an infection with *Ascaris suum* on the intestinal microbiota of the host

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Introduction

Infections with soil-transmitted helminths are a major health problem. The roundworm *Ascaris lumbricoides* in humans and *Ascaris suum* in pigs is the most prevalent internal macroparasite. In pigs, *A. suum* infections cause a lag in the increase of weight and growth and subsequently lead to economic losses. Since *Ascaris* resides in the intestine, it is hypothesized that there is a crosstalk between the worms and the gut microbiota, in that way that an infection with *Ascaris* could influence the composition of the gut microbiota.

Materials and Methods

Twenty-four female and male pigs of 10 weeks old were included. Twenty of these pigs were infected with *A. suum* eggs. The remaining 4 pigs served as uninfected naive control animals. The 20 pigs in the infected group received 3 doses of 300 fertilized *A. suum* eggs, on 3 consecutive days. At day 54 post-infection, all pigs were euthanized. The number of adult worms present in the small intestine was counted. Infected pigs were classified as either pigs with high worm count, or pigs with very low to zero worm count. In both of these groups, 5 pigs were randomly selected for further procedures. Intestinal contents of all of the pigs were sampled at the colon, 30 cm distal from the junction of the caecum and proximal colon. The samples were snap-frozen and preserved at -80°C until further processing for microbiota and short chain fatty acid (SCFA) determination. For the characterization of the microbiota, bacterial DNA was extracted from the colon contents and 16S rRNA taxonomic profiles were determined.

Results

In pigs, most *A. suum* larvae are expelled after 2 or 3 weeks of infection, which led to the subdivision of infected animals into a group with and a group without adult worms. When analyzing bacterial diversity, one has to take into account two different factors, species richness and species evenness. Richness expresses how many different species are found. Evenness shows how many different individual bacteria are found in the species groups. Control animals displayed a higher bacterial diversity than infected animals with high

worm burden, resulting from statistically significant higher species richness and evenness in the control group. Bacterial diversity of infected animals with zero worms was found in between the control and infected animals, without being significantly different from neither of the 2 groups. When performing further statistical analysis at the level of phylum, family, genus and species, it became clear that between the subgroups of infected animals with and without worms, there were only significant differences found at genus and species level. The main differences were seen in *Prevotellaceae* populations, with *Prevotella* as the most prominently differing genus. Furthermore, SCFA analysis showed that 3 weeks post-infection, total SCFA concentrations were increased, compared to control animals. The SCFA's responsible for this, were propionate and butyrate, with a significant increase in infected animals, and acetate with a trend towards higher concentrations. Interestingly, the increase in concentrations was higher in pigs with less worms.

Conclusions

Significant differences were found between control and *A. suum* infected animals concerning bacterial diversity and SCFA concentrations. Interestingly, when combining these data, links between bacterial genera and SCFA production can be found. The genera *Megasphaera* and *Prevotella*, which are more represented in infected animals, are known to be associated with SCFA production. Also, the presence of adult worms is not indispensable for the observed effect on SCFA concentrations. This might mean that only the passage of larvae is already causing alterations. The communication between intestinal worms and the microbiota, might also have an important influence on the host's immune response.