Creation of a Mouse Model with Equinized Gut Microbiota

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Introduction

- The importance of gut microbiota (GM) in host health has long been appreciated
  - Atypical variation of GM has been linked to various maladies such as inflammatory bowel diseases (Crohn's Disease and ulcerative colitis), obesity, and cancer.
- Mice are optimal models for the study of GM
  - Inbred strains allow for control of genetic factors
  - They are cost-effective to house and breed

Study Objectives

- The objective of this study was to create an 'equinized' mouse colonized by equine GM
  - Such a model will be useful for the economical study of equine diseases such as colic and metabolic syndrome

Methods

- 16 FVB/NJ female mice were obtained from the Jackson Laboratory
- Mice were treated with antibiotics [Neomycin (1 g/L), Vancomycin (0.5 g/L), Ampicillin (1 g/L), Metronidazole (1 g/L)] in drinking water for 5 consecutive days
- Mice were subsequently gastric gavaged with an equine fecal-material in a nuclease-free water slurry for 3 days
- Fecal samples were collected on three separate dates:
  1. Pre-antibiotics
  2. Post-antibiotics
  3. Post-gavage
- DNA was extracted from fecal samples and subjected to 16S rDNA sequencing and analysis

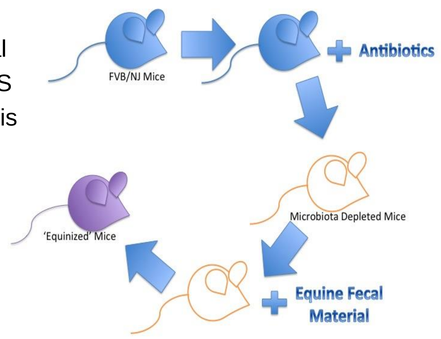

Figure 1. Depicts the depletion of the natural GM of the mouse antibiotics and the subsequent transfer of equine fecal material.

Results

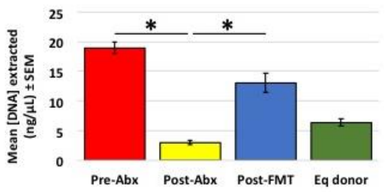

Figure 2. Bar chart showing the amount of DNA extracted from fecal samples at each time point. Asterisks indicate $p < 0.05$, ANOVA on ranks; equine donor sample not included in comparison due to $n = 3$ replicates from the same horse.

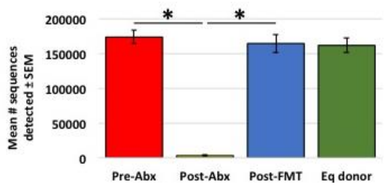

Figure 3. Bar chart showing the mean number of sequences detected from fecal samples at each time point. Asterisks indicate $p < 0.05$, ANOVA on ranks; equine donor sample not included in comparison due to $n = 3$ replicates from the same horse.

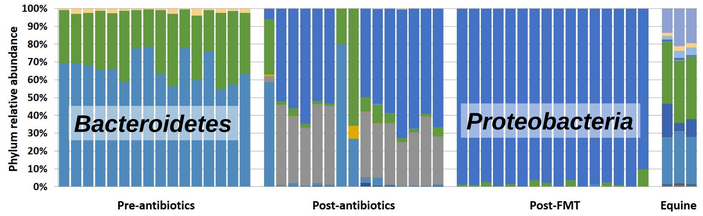

Figure 4. Microbiota at the phylum level from 16 mice at three different time periods (pre-antibiotics, post-antibiotics, post-fecal microbial transfer (FMT)) as well as the composition of a single equine sampled on three separate days.

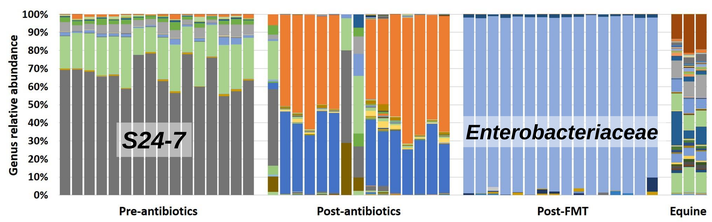

Figure 5. Microbiota at the genus level from 16 mice at three different time periods (before antibiotics, post-antibiotics, post-FMT) as well as the composition of a single equine sampled on three separate days.

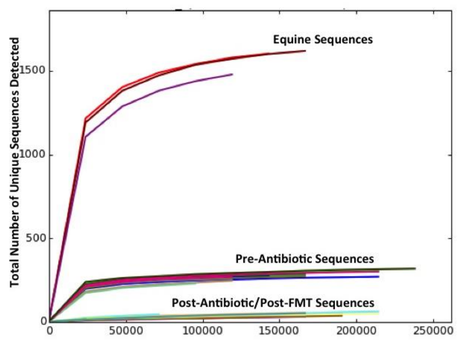

Figure 6. Rarefaction curves for pre-antibiotic sequences, post-antibiotic sequences, post-FMT sequences, and equine sequences to determine richness.

Results

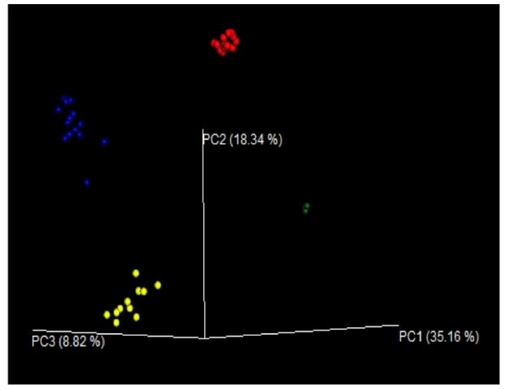

Figure 7. Principal component analysis (PCA) reveals distinct clustering of individuals from the four study groups demonstrating similarity among individuals within groups and differences between groups. PCA generated with Emperor software.

Conclusions

- Pre-antibiotic analysis showed typical murine GM
- Post-antibiotic analysis confirmed marked depletion of endogenous murine GM and loss of richness with remaining bacteria representing those found in food (e.g. *Streptophyta*/*Zea*)
- Post-FMT analysis showed GM dominated by *Enterobacteriaceae* (up to 99% of GM)
- FMT did not result in a reconstitution of the equine GM
- This model may still be useful for studying other diseases
  - *Enterobacteriaceae are* associated with many maladies such as Inflammatory Bowel Diseases and septicemia.

Future Directions

1. Determine sustainability of these *Enterobacteriaceae* levels
2. Breed mice to create a sustainable line
3. Repeat experiments to ascertain reproducibility of results

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