Peripheral Immune Modulation, Temporal Dynamics, and Community Shifts of Canine Gut Microbiome after Fecal Microbiota Transplantation

Mary Ann Lee, Bart Weimer, Agostino Buono, Maria Questa, Jan Suchodolski, Sina Marsilio
Department of Medicine and Epidemiology, UC Davis School of Veterinary Medicine, Davis, CA;
Texas A&M University Veterinary Medicine & Biomedical Sciences, College Station, TX

Background

- Fecal Microbiota Transplantation (FMT) is a therapeutic manipulation of the gastrointestinal microbiome by transplanting a healthy donor's stool sample to a recipient's intestinal tract to shift the gut microbial community and establish normobiosis
- Most clinical trials exploring FMT efficacy are conducted in patients with existing dysbiosis, making it difficult to understand safety and mechanisms of FMT engraftment across clinical uses
- A previous pilot study has shown successful engraftment of donor-derived strains over a period of a year in three healthy human recipients, but a similar study has not been conducted in veterinary medicine
- Majority of microbiome studies are conducted with 16S sequencing, resulting in limited taxonomy resolution
- This pilot study aims to characterize community shifts in intestinal microbiome of healthy dogs following a single FMT administration as a rectal enema over 28 days

Hypothesis

Fecal Microbiota Transplantation in healthy dogs will be clinically safe, with no significant changes in the peripheral immune system, and result in a stable engraftment of the donor dog's gut microbiome over a 28 days' observation period

Methods

- Spontaneously passed feces from 2 canine donors were collected, processed with 0.9% saline, glycerol added to final concentration of 10%, and frozen at -80C until use
- 10 Healthy dogs received 5g/kg rectal enema FMTs
- Serum for CBC, chemistry, C-reactive protein (CRP), cytokines, and feces for dysbiosis index (DI) and sequencing collected on days 0, 1, 4, 10, 28 post-FMT
- Clinical surveys (modified CIBDAI) from owners included: attitude/activity, appetite, vomiting, stool frequency, mucous or blood in feces, fecal score using Nestle Purina Fecal Scoring System (7-point scale; 1 = constipation, 2-3 ideal, 4-7 diarrhea)
- Whole metagenomic shotgun sequencing of gut microbiome recipients sequenced using Illumina HiSeq XTen using PE150 protocols and metagenomic library assembled using Kraken v.2 was used identified species level resolution of microbiome for each sample
- Statistical analysis included Shapiro Wilks normality tests and mixed effects model or one way analysis of variance (ANOVA) based on dataset

Figure 1.

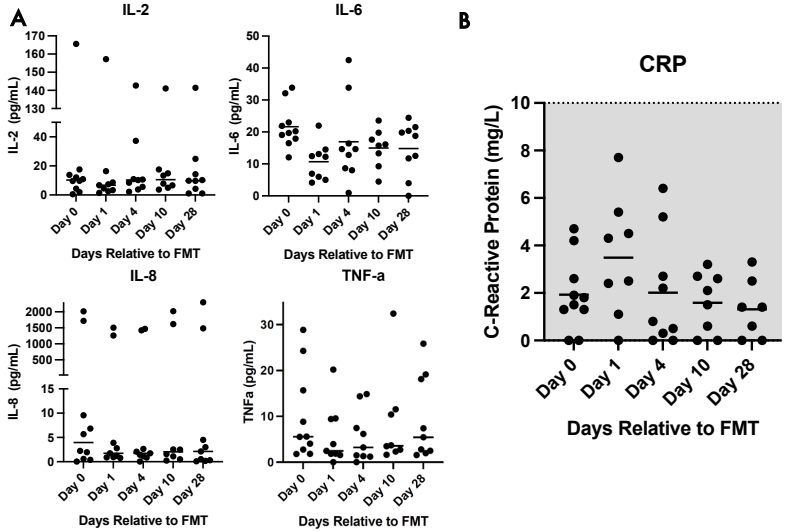

Figure 1: Cytokine levels of IL-2, IL-6, IL-8, and TNF-a (1A), and C-Reactive Protein (CRP; 1B) levels following FMT in healthy recipients. Inflammatory cytokines measured were not increased post-FMT. All values of CRP remained within the reference range.

Figure 2.

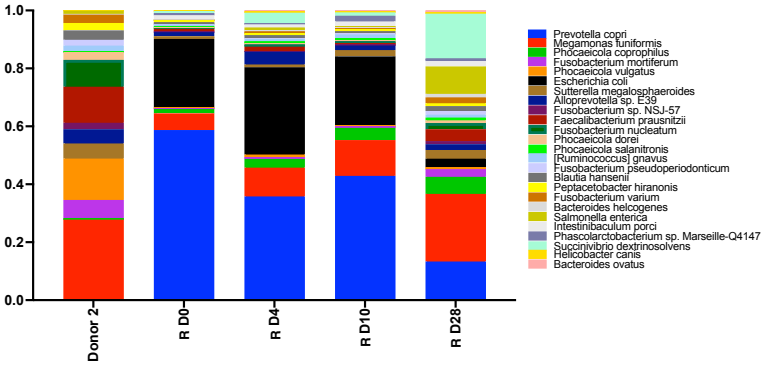

Figure 2: 24 most abundant bacterial species sequenced in a single donor (Donor 2) and a single recipient (R) over days 0, 4, 10, and 28 in proportion. Note the changes in the microbiome over time to resemble the donor. For instance, *Escherichia coli*, not present in the donor's stool sample but abundant in the recipient's samples at baseline, decreases to a smaller percentage, indicating convergence of the recipient's stool sample towards the donor. Also, abundance of *Faecalibacterium prausnitzii* was high in the donor and low in the recipient at baseline, but increased at recipient's day 28 sample.

Results

- One-time, rectal enema FMT was well tolerated by all 10 healthy canine recipients with no known major side effects
- 3 recipients with vomiting and 4 with transient diarrhea were reported during the 28 days following FMT, with a case of diarrhea and a case of vomiting attributed to dietary indiscretion
- Patients had normal appetite and no mucous or blood in stool
- All CBC and serum biochemistry values were not significantly different between days 0, 1, 4, 10, and 28
- Initially significant p-values for anion gap and bicarbonate adjusted for false discovery rate using Benjamini-Hochberg were not significant ($p = 0.13$, $p = 0.18$ respectively)
- Cytokines (IL-2, IL-6, IL-8, TNF-a), CRP, and DI values were not significantly different between days
- 7093 species were sequenced using WGS (89% bacteria)
- 40 different species of bacteria present in the donor but not initially present in the recipient sample were seen in days 1, 4, 10, 28 and defined as engraftment following FMT

Discussion and Future Aims

- One-time rectal enema FMT in healthy dogs is relatively safe
- FMT in healthy dogs did not lead to significant changes in the safety and peripheral immune system markers measured in the study (CBC, chemistry, cytokines, CRP)
- Engraftment of 40 different bacterial species were seen that were not present in the recipient before FMT
- Using StrainFinder in R, we will resolve sequences to bacterial strain level to compare pre- and post-FMT shifts as well as identify engraftment factors of FMT
- Multivariate statistics for population diversity and taxonomy will be performed on current data
- Future study will be conducted in dogs with chronic enteropathy

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Marsilio Laboratory; Sina Marsilio, smarsilio@ucdavis.edu

Mary Ann Lee; mjrlee@ucdavis.edu, UC Davis School of Veterinary Medicine c/o 2024