**INTRODUCTION**

Idiopathic chronic diarrhea (ICD) is a common cause of morbidity in captive rhesus macaques for which the underlying pathogenic mechanisms remain poorly understood. At the CNPRC, the incidence of ICD in each year’s birth cohort is ~5% with a peak onset and diagnosis in animals 9 months to 3 years of age and often resulting in euthanasia. Early life GI dysbiosis including as a result of antibiotic administration has a distinct role in the maturation of the immune system and may cause the development and persistence of ICD. Recent research in human and monkey subjects has shown that restoring native populations of gut microbial communities may be useful in managing chronic diarrhea. Normal, healthy digestive tracts with these populations of bacteria prevent the habituation by pathogenic bacteria. Dysbiosis following GI disease and diarrhea or antibiotic treatment may be treated with induction of normal flora using fecal bacteriology as shown in a study with macaques showing post-treatment clinical improvement.

In a preliminary study done at the CNPRC, a 6 day course of oral symbiotic (probiotic and prebiotic) treatment has been shown to clinically improve fecal consistency scores. We hypothesize that this administration of symbiotic treatment will beneficially alter gut microbial communities in ICD patients.

**MATERIALS AND METHODS**

**Case definition.**

Case animals in this study are selected based on their diagnosis of ICD, defined as diarrhea for 45 out of 90 days for indoor animals and diarrhea for 45 out of 90 days or having been admitted to the hospital 3 or more times in a year for outdoor animals. Also required are:

- Three negative rectal cultures for common enteric bacterial pathogens
- One negative test for enteric parasites
- Single negative IFA for Cryptosporidium spp. and Giardia spp.
- No response to empirical treatment with tetracycline, metronidazole, or prednisone.

**Animal selection.**

Animals were selected based on a diagnosis of ICD.

**Randomized controlled trial.**

- **Symbiotic treatment group:** Symbiotic treatment consisting of probiotics Enterococcus faecium, Lactobacillus acidophilus, Lactobacillus casei, and Lactobacillus plantarum combined with prebiotic soluble fiber psyllium husk was administered orally BID as a sandwich to 5 ICD patients for 5 days.
- **Placebo group:** A pudding sandwich was administered to 5 ICD patients, age and gender matched to symbiotic treatment group members.

**Study duration.**

10 animals were randomly assigned to the two groups for a 18 day study. Animals were moved into the hospital at least 5 days before beginning treatment. Treatments were given for 5 days and blood and stool samples were collected on day 0 and day 5 of treatment. Fecal consistency scores were collected for all days. Some animals were taken off study before the full 18 days for therapeutic intervention.

**Assessment of clinical signs.** Fecal consistency score (FCS) were recorded daily. Scores were ranged from 1 to 4 (1-normal stool, 4-liquid diarrhea). Scores are assessed as stated in the following table:

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Well-formed normal</td>
</tr>
<tr>
<td>1.5</td>
<td>Normal to semi-solid</td>
</tr>
<tr>
<td>2</td>
<td>Semi-solid</td>
</tr>
<tr>
<td>2.5</td>
<td>Semi-solid to liquid</td>
</tr>
<tr>
<td>3</td>
<td>Liquid</td>
</tr>
</tbody>
</table>

*Table 1: Stool quality was evaluated daily, and the stool consistency was scored using scaled scores.*

**16S rRNA Sequencing**

Fecal samples were taken from each patient before and after symbiotic or placebo treatments. Microbial DNA was harvested using MO BIO’s PowerSoil®-DNA isolation Kit and analyzed by 16S rRNA sequencing to identify bacterial species and changes in gut microbiota.

**RESULTS AND DISCUSSION**

**Clinical.**

The fecal consistency scores of the two groups were significantly different when comparing the average scores of the last 3 days of treatment (p=0.044, Figure 1) (boxplot). Figure 2 shows the average FCS of each group over the study period and indicates a drop in FCS during the treatment period (days 0-5) which correlates with clinical improvement.

**Sequencing Analysis.**

Gut microbiota identification was done by 16S rRNA gene sequencing in pre- and post-treatment fecal samples collected in Symbiotic and Placebo groups. Principal component analysis shows a trend towards separation between Symbiotic and Placebo groups in terms of microbiota changes following treatment (Figure 3), however, this difference was not significant due to the small sample size (n=3). The sequencing results also indicate that symbiotic treatment decreases the number of observed species and diversity in terms of richness and evenness. Conversely, the Placebo group had an increase in these parameters after the treatment period (Figure 3). This is the opposite of what we would expect from previous studies that suggest a lower diversity actually leads to a poorer clinical state.

**Specific changes in bacterial families for both groups is shown in Figure 5. Ruminococcaceae had a positive correlation with FCS suggesting that a lower abundance of this bacterial family leads to an improved clinical outcome (Figure 6). This was consistent with our results as the Symbiotic group exhibited decreases in abundance of Ruminococcaceae and FCS following treatment. In mice with experimentally induced colitis, this family has been shown to increase in relative abundance. In humans, some Ruminococcaceae species are also increased in Chron’s disease. Cats with chronic diarrhea have been shown to have significant decreases in Veillonellaceae and this family is increased in abundance in our study as a result of treatment. Prevotella is in gut is linked to inflammation and has been linked to loose stool in humans if their gut microbiota is of the Prevotella enterotype, but is increased in our study by symbiotic treatment**

**ACKNOWLEDGMENTS**

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