# Characterization of Gut Microbiome in a Porcine Model of Traumatic SCI

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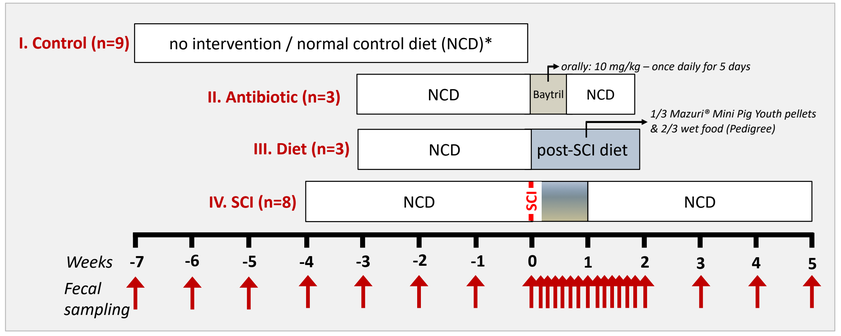
## Role of the gut microbiome in spinal cord injury

In recent years, the complex interplay of the microbiome, particularly the gut microbiome, and the central nervous system (CNS) has become a growing area of research. Emerging evidence from experimental models in stroke and traumatic brain injury (TBI), as well as spinal cord injury (SCI), point towards a potential bidirectional communication: (1) changes in the gut microbiome after CNS-trauma, and (2) the influence of this dysbiosis on disease pathogenesis. In addition, experimental interventions on the gut microbiome have shown promise in improving functional outcomes in murine models of SCI. This commensal relationship between changes in gut microbiome and human health/disease provides an exciting new frontier in SCI research.

With the increased interest in the microbiome's role in the pathogenesis of SCI, **we aimed to characterize the baseline gut microbiota and changes that are associated with SCI, using our pig model.** Hence, evaluation in a large animal model (e.g. pigs) with very similar gut microbiomes to that of humans, is a crucial step in generalizing this knowledge to humans.

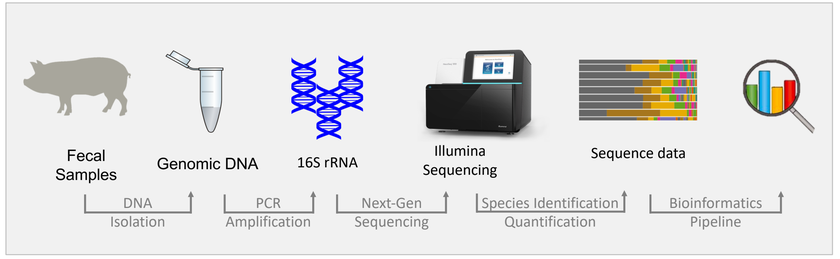
## How the gut microbiome changes in a pig model of SCI

For this study, female Yucatan miniature pigs were used. Four groups of animals: (1) Control, (2) Antibiotic, (3) Diet, and (4) SCI), which were used to evaluate the effect of SCI on the composition of the porcine intestinal microbiome while trying to control for dietary changes and antibiotic treatment post-injury. SCI was induced by a 50-gm impactor dropped from a 20-cm height followed by 5 minutes of compression (Lee et al., J Neurotrauma 2013, 30(3):142-59. doi: 10.1089)




** Normal control diet: 3/3 Mazuri® Mini Pig Youth pellets & 1 tbls wet food (Pedigree)*

## Characterization of the porcine gut microbiome using 16S rRNA Genome Sequencing




To identify the gut microbial in our pig model, fecal samples were collected and prepared for bacterial 16S ribosomal RNA (rRNA) gene sequencing. Genomic DNA was extracted from 288 fecal samples. PCR was used to amplify the 16S rRNA gene from all the different chromosomes, resulting in a pool of 16S sequences representing different species of bacteria present, approximately in proportion to their composition in the porcine feces. Data was quantified and analysed using 16S rRNA gene bioinformatics pipeline.

## Taxonomic characterization of baseline porcine gut microbiome

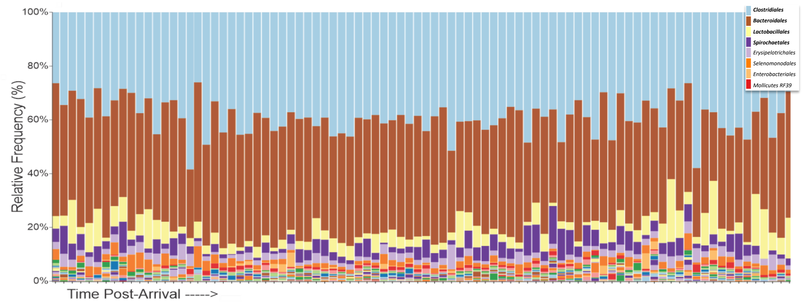



**Figure 1.** Relative frequency (%) of all bacterial phylogenetic orders present in fecal samples from Yucatan minipigs (n=9, 93 samples). Taxonomic distribution of the numerically abundant bacterial orders derived from the pig metagenomes revealed that Clostridiales , Bacteroidales , Spirochaetales , and Lactobacillales were the top four most abundant bacterial groups. Bacteriodales representing the largest portion and accounting for ~40% of pig fecal samples, Clostridiales for ~35%, Lactobacillales for ~5%, and Spirochaetales for 5%.

## Relative frequency of the four major bacterial orders in the gut following antibiotics treatment and dietary change

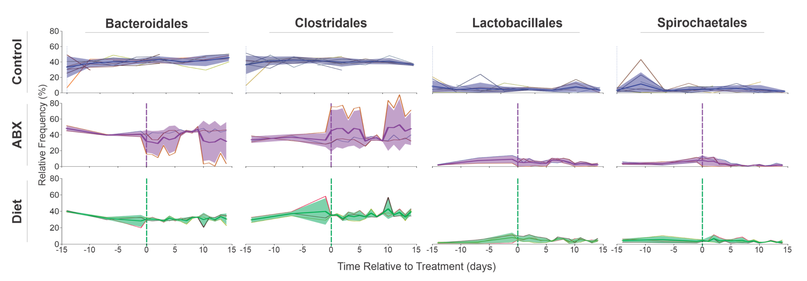



**Figure 2. (A)** Relative frequency (%) of Clostridiales , Bacteroidales , Spirochaetales , and Lactobacillales in pig fecal samples were generally consistent even across different animals from arrival at the animal facility to 30 days after arrival (n=9). **(B)** During and after antibiotic (ABX) treatment, the relative abundance of Bacteroidales decreased and corresponded with increased Clostridiales in two out of 4 animals. Additionally, bacterial depletion of Lactobacillales and Spirochaetales was observed after antibiotic treatment. **(C)** Dietary change had little impact (n=3, 45 samples). Mean ± SD are represented as a bold line and shaded region.

## SCI: Relative frequency of the four major bacterial orders in the gut following injury

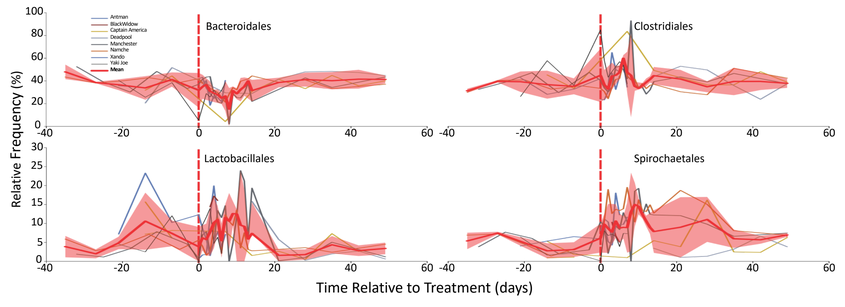



**Figure 4.** Fecal samples of SCI pigs showed a time-dependent effect of SCI on gut microbiome composition. Bacteroidales and Clostridiales, the two most prevalent bacterial taxa in gut, were inversely regulated early after SCI (<14 days): Bacteroidales decreased as a function of time, while Clostridiales increased. In addition, minor taxa, including Lactobacillales, and spirochaetales, were increased. Mean ± SD are represented as a bold line and shaded region, respectively.

## Comparison of alpha diversity (species evenness and richness) following antibiotics treatment, dietary change and SCI

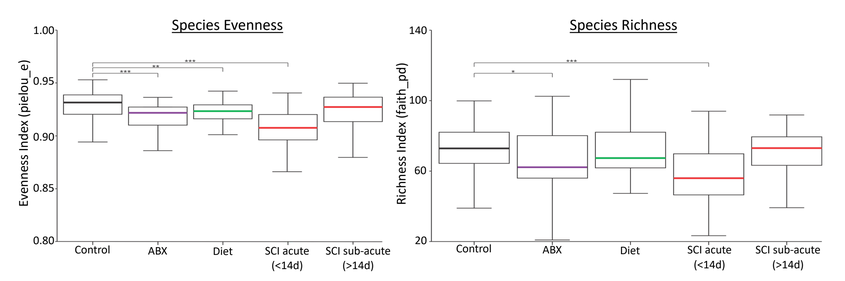



**Figure 5.** Species richness is the number of different species present in a sample whereas evenness compares the similarity of the population size of each of the present species. Species evenness significantly decreased following antibiotics (ABX) treatment, dietary change and during the acute stage after SCI. Species richness decreases in ABX and acute SCI. Differences assessed using Kruskal-Wallis pairwise comparison. *=p<0.05; **p<0.01; ***p<0.001

## SCI: Taxonomic characterization of porcine gut microbiome following injury

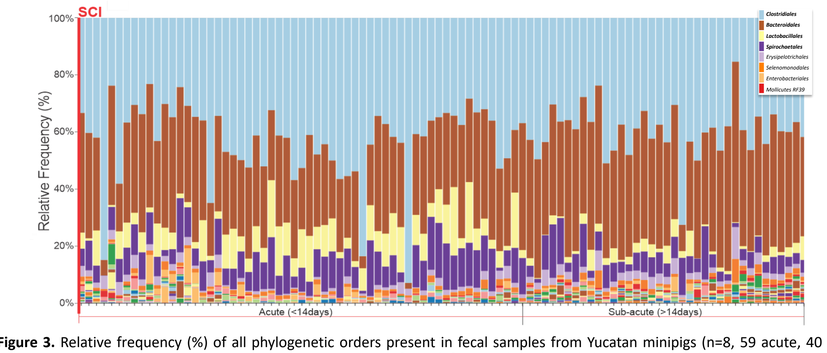



**Figure 3.** Relative frequency (%) of all phylogenetic orders present in fecal samples from Yucatan minipigs (n=8, 59 acute, 40 sub-acute samples). Days relative to treatment increases from left to right. Clostridiales, Lactobacillales, Spirochaetales populations seemed to increase in abundance following SCI – primarily in the acute stage (<14 days).

## CONCLUSION AND DISCUSSION

- The gut microbiome of the Yucatan minipig appears fairly stable before any treatment, even across different animals, both in terms of the species present and their relative frequencies.
- The dominant phylogenetics orders which occupy the Yucatan microbiome differ from human models such that there is a greater proliferation of Coriobacterales and Selenomodales orders in the human gut (Almeida et al. 2019).
- Unlike a recent study in the murine model which showed that Bacteroidales and Clostridiales populations continued to decrease and increase, respectively, three weeks post-injury, the relative frequency of Bac/Clos populations in Yucatan minipigs returned to baseline levels ~2-3 weeks post-SCI (Kigerl et al. 2016), which is more similar to the trend observed in human subjects following antibiotic treatment (Dethlefsen et al. 2008).
- Human SCI patients were shown to have lower levels of butyrate-producing bacteria such as Pseudobutyrivibrio, Dialister and Megamonas genera, which may play a role in microglia-mediated neurotoxicity following SCI (Gungor et al. 2016).