DISCORDANT MOUSE ENGRAFTMENT OF THE GUT MICROBIOTA FROM NEONATES DISCORDANT FOR DELIVERY MODE

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ABSTRACT OF THE THESIS

Discordant Mouse Engraftment of the Gut Microbiota from Neonates Discordant for Delivery Mode

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Caesarean section (CS) is an increasingly common procedure by which the baby is born sterile into the air of the operating room, bypassing the normal exposure to maternal microbiota along with antibiotic exposure leading to altered gut microbiota composition based on birth mode. CS born do not acquire the normal microbiota inoculum at birth, and their microbiota has been shown to be abnormal. We hypothesize that the differences in the human neonatal microbiota lead to different engraftment in germ free mice.To test thishypothesiswe aimed to determine microbiota engraftment in sterile mice inoculated with human baby feces discordant to birth mode and the effect of high fat diet (HFD) on the growth and microbiome development. To accomplish these aims we transferred feces from 2 days old human neonates born vaginally (VD) or by CS into 32 germ free (GF)female mice (n=16 in each group)4-6 weeks of age. After 4 weeks post gavage, half of the mice of each group (n=8) were given high fat diet for 5 weeks. Body weight and fecal microbiota structure of the mice were examined at 3-, 5-, 7-, and 9-weeks post gavage. Microbiota richness, beta diversity and discordant taxa were determined using QIIME2 and LEfSe.

The results showed that ~300 ASVs were present in the human feces from human neonates, with higher richness in babies born by CS (181 ASVs) than in VD infants(100 ASVs). Bacteroides dominated the feces of babies born by VD while Citrobacter dominated the feces from babies born by CS.

The neonatal human inocula showed poor engraftment in mice: only 7-17 of the ~300 ASVs in the human feces engrafted in mice. Contrary to what was expected with the higher richness in the feces of CS born babies, mice transferred the feces from CS born showed lower engraftment (7 ASVs) than mice receiving feces from VD infants (17 ASVs).LefSe analysis showed that microbiota in mice receiving the VD inoculum was overrepresentedby Escherichia-Shigella, Pantoea, Enterococcus, and Bifidobacterium, while Citrobacterkoseri was dominant in mice receiving CS inoculum. HFD did not appear to affect the microbiota, though it did result in phenotypic differences with mice consuming HFD gaining more weight. The results suggest that the microbiota of human neonates engrafts poorly into GF mice, and that there are marked differences in engraftment of the feces of neonates by birth mode.

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INTRODUCTION

Caesarean section (CS) is an increasingly common procedure, with rates increasing worldwide particularly faster in low resource countries (Betrán et al., 2016). In CS, babies are born sterile into the air of the operating room, bypassing the normal exposure to maternal microbiota along with antibiotic exposure. These babies have exposure to the wrong set of microbes, and have exposure to antibiotics (Perez-Muñoz et al., 2017, Theis et al., 2019), and show altered microbiota communities compared to VD born(Mueller et al., 2014, Martin et al., 2016, Shao et al., 2019), acquiring at birth maternal skin communities rather than maternal vaginal communities (Dominguez-Bello et al., 2010).

In humans, C-sections are typically done under the effects of antibiotics, which can exert compounded effects on the infant bacterial colonization, early life antibiotics alter gut microbiota community with even miniscule applications resulting in alterations of taxonomic composition (Cho et al., 2012). The consequences have been suggested to include colonization of opportunistic pathogens (Shao et al., 2019), as well as long term consequences of bad education and programming of the immune system. Early microbiota shifts due to CS birth and antibiotics have been linked to numerous diseases and disorders such as obesity (Azad et al., 2014), respiratory infections such as asthma and laryngitis (Reyman et al., 2019a, Kristensen & Henriksen, 2016), and gastroenteritis (Kristensen & Henriksen, 2016).

High fat diet (HFD) also alters the microbiome structure when given tomice (Daniel et al., 2014, Hildebrandt et al., 2009, Salonen & de Vos, 2014, Turnbaugh et al., 2008, Turnbaugh et al., 2009) and phenotype of mice (Wang & Liao, 2012). In our pilot study, introduction of

HFD was used to determine the effect of HFD on the different engrafted microbiota, and the effect on body weight gain.

The microbiome can be studied using either shot gun metagenomics or, to gather taxonomic information on compositiopn and relative abundances, the 16S rRNA gene can be amplified and sequenced.16S rRNA encodes a ribosomal subunit which is neccesary for protein assembly, and holds a taxonomic signature, being highly conserved withinspecies and different between species. Analysis of conserved regions can thus be used to identify bacteria and archaea in the microbial community of samples (Johnson et al., 2019).

HYPOTHESIS

We hypothesize that the differences in the human neonatal microbiota are reflected in different engraftment in germ free mice.

AIMS

To test this hypothesis, we propose the following aims:

1- Determine microbiota engraftment ex-sterile mice inoculated with human baby feces discordant to birth mode

2- Determine the effect of high fat diet (HFD) on microbiome and growth in mice with different microbiota

EXPERIMENTAL DESIGN AND METHODS

Mouse experiment

We used 32 Germ Free C57BL/6NTac female mice housed in groups of maximum 6 animals per cage. Mice were 4-6 weeks of age and given 3 days of acclimation prior to commencement of the experiment. Mice were separated into 2 groups of 16 mice with one group gavaged with inocula of pooled feces from four 2-day old, human neonates born vaginally and the other gavaged with inocula of pooled feces from four 2day old, human neonates born by cesarean section. All animals were fed standard chow diet (Teklad Global Soy Protein-Free Extruded Rodent Diet 2020SX) for 4 weeks post gavage after which half of the mice of each group (n=8), were transferred to high fat diet (Teklad TD.08811) for the remaining 5 weeks of the experiment.

Outcomes

Body weight: mice were weighed at time points 3-, 5-, 7-, and 9-weeks post gavage.

Fecal microbiota: Fresh mouse feces was collected at 3-, 5-, 7-, and 9-weeks post gavage, in 2ml cryotubes, and stored at -80 degrees Celsius.

Fecal DNA Extraction, Amplification, and Sequencing

Mouse feces were treated using the MoBio Powersoil Kit with modified protocol as described by Earth Microbiome Project (Marotz et al., 2017) to extract DNA and the highly conserved V4 region of the 16S rRNA gene was then amplified using PCR with barcoded primers (Bukin et al., 2019). DNA samples were then sent out to an outside company to sequence using the Illumina MiSeq platform using a paired end technique in order to determine the

phylotypes present. Quality filtered reads were de-multiplexed and quality filtered with QIIME2 and clustered using Silva132 as reference taxonomy.

Microbiota Analysis

Microbiota richness, beta diversity and discordant taxa were determined using QIIME2 microbiome bioinformatics platform (Bolyen et al., 2019). After processing, microbiota richness was determined in alpha diversity graphs of microbiota richness and community compositions. All statistical tests were two sided and a p value of .05 was considered statistically significant. Beta diversity, comparing microbiota community structure between samples/individuals, was plotted with PCoA graphs graphed using UNIFRAC distances (Lozupone et al., 2011) with statistical significance done using PERMANOVA. Discordant taxa were identified using LEfSe analysis (Segata et al., 2011).

RESULTS

a) Human neonatal fecal inoculum

The human inoculum consisted of pooled feces from 8 2-day old infants, 4 born by VD and 4 by CS. Human inocula segregated apart from mouse feces **(Fig 1A),** and CSbabies showed higher alpha diversity than VD neonates (100 ASVs versus vs 181 ASVs, respectively) **(Fig 2A, Table 1)**.

Compositionally, the two fecal inocula used to conventionalize the mice were different: Bacteroides dominated the feces from VD neonates, while Citrobacter dominated in the feces from CS bornalong with the notable presence of Bifidobacterium in VD inoculum **(Fig 2B-C)**.

b) Mouse microbiota diversity

Alpha diversity-The microbial communities from the infants discordant by birth mode led to different engraftment in mice. Mouse microbiota segregated significantly by inoculum (CS or VD), and time post gavage, though not by diet (no effect of HFD), at least during the 9 weeks of the experiment (ANOSIM p=.001) (**Fig 1A-C)**.

Neonatal human inocula had only 7-17 ASVs of the ~300 ASVs in the human feces engrafting in mice**(Fig 2, Table 1)**. Mice receiving feces from VD infants showed engrafted communities withgreater alpha diversity than those in the VD group, and this trend was unaffected by time and diet: 17 vs 7 ASVs engrafted in the mice transferred the feces from VD and CS human babies, respectively (**Fig 2A, Table 1)**. Other measures of alpha diversity

reflect increased human inoculum diversity when compared to engrafted mouse samples **(Fig 3A-D).** Simpson and Shannon indices which represent diversity when accounting for species relative abundance and evenness respectively show that while diversity did not differ significantly by diet, diversity in mice consuming normal chow increased over time, however the results may be skewed by the dominance of singular taxa in mice samples **(Fig 2B, Fig 3C-D).**

Beta diversity-The differences in beta diversity were primarily along the primary principle coordinate, which showed that the mouse communities separated by source of inoculum (CS or VD), consistently in time, and independent of diet (**Fig 1D)**.High fat diet did not appear to significantly affect the microbiota **(Fig 1, Fig 2).**

Group-discordant bacterial taxa-The microbiota which engrafted in the mice also differed from that in the human inoculum: Bacteroides, dominant in the feces of vaginally born human neonates did not engraft in mice; Citrobacter, which was detected in both human inocula, completely dominated the gut of mice conventionalized with feces from CS-born, but not in VD born infants, which were in turn dominated by Escherichia-Shigella, with the notable presence of Enterococcus and Lactococcus (**Fig 2B)**.

Engrafted taxa present in the rare taxa (<1%) were similar between CS and VD mice with the notable exception Bifidobacterium which engrafted in mice receiving the inoculum from the VD human babies, and not those in CS group**(Fig 2C).** Rare taxa made up a significantly Lower proportion of the mouse gut communities, compared to the human inocula **(Fig 4).**

LEfSE analysis of 3 weeks post gavage showed that Citrobacter koseri was the overrepresented species which solely dominated the mice that received inocula from CS born human babies, while E. coli dominated VD mice communities along with the notable representation of Bifidobacterium, Lactococcus, and Pantoea(**Fig 5A)**.The dominant taxa in both groups, E. coli in the VD and C. koseri in the CS, engrafted in high proportions and remained high in the weeks following the inoculation, while Enterococcus increased and Bifidobacterium decreased with time in the VD group and Pantoea was no longer significantly overrepresented in VD mice past 3 weeks post gavage **(Fig 5B-E, Fig 6)**.Based on the LEfSe cladograms, Firmicutes and Actinobacteria were associated with VD inoculated mice while Proteobacteria was associated with CS inoculated mice with the sole exception of E. coli **(Fig 6)**.

c) Mouse growth

Body weight was measured before inoculation and at 3, 5, 7, and 9 weeks post inoculation and averaged. Mice were at juvenile age when inoculated and were considered fully developed (at 4-6 weeks of age) **(Fig 7A).** Despite randomization of the mice to receive the feces from the human babies born by VD or CS, the CS inoculated mice were smaller than VD inoculated mice at the time of inoculation **(Fig 7A).**

Total body weight growth of CS mice were significantly higher than VD mice at all time points, however the difference in mice ages and significantly lower starting body weight renders the data on mouse growth untrustworthy **(Fig 7B).**

As expected, high fat diet resulted in significantly greater body weight when compared to the normal chow counterparts by 9 weeks post gavage **(Fig 7).**

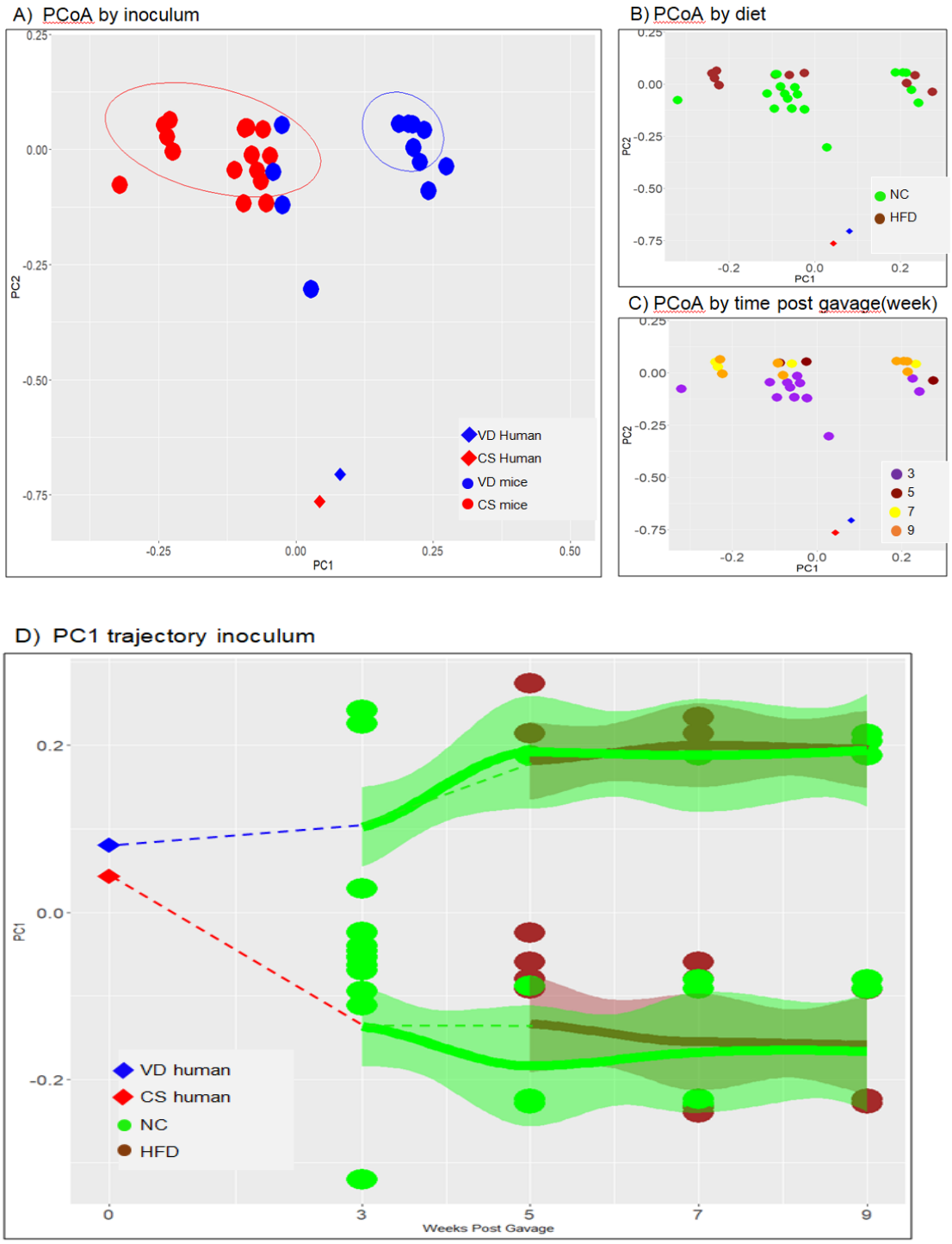

Fig1. Beta diversity of the mouse microbiota after humanization of GF mice with neonatal feces. A= PCoA by inoculum; B= PCoA by diet; C= PCoA by weeks post gavage; D= PC1 trajectory of the microbiota. Inoculum is represented by diamonds, mouse samples are represented by dots. VD=Blue, CS=Red, Normal Chow=Green, High Fat Diet=Brown. Mouse microbiota segregates by inoculum, and time post gavage, not by diet, at least during the 9 weeks of the experiment. Inoculum differences are sustained in time.

Table 1. **Number of reads and proportions of human bacterial taxa engrafting in mice at genus level.** CS inoculum had greater number of genera present compared to VD inoculum 181 vs 100.Diversity is lost with engraftment into mice with of the hundred genus present in VD inoculum, only 17 engraft with only 2 taxa dominating while of the 181 taxa present in CS inoculum, only 7 engraft. CS inoculated mice are dominated by a single taxa compared to VD inoculated mice which has slightly greater diversity.

	Total # of Reads	Total # of Genus	Genus above 1% Rel. Abu.	Genus between .5% and 1% Rel. Abu.	Genus less than .5%
VD Inoculum	38333	100	5	4	91
VD NC Week 3	34114	17	2	1	14
VD NC Week 5	37469	6	2	1	3
VD NC Week 7	37589	6	2	1	3
VD NC Week 9	37905	7	2	1	4
VD HFD Week 5	35419	12	3	0	9
VD HFD Week 7	40085	10	3	0	7
VD HFD Week 9	39715	9	2	0	7
CS Inoculum	37148	181	8	13	160
CS NC Week 3	20359	7	1	0	6
CS NC Week 5	31350	8	1	1	6
CS NC Week 7	24238	5	1	1	3
CS NC Week 9	26635	4	1	1	2
CS HFD Week 5	28322	6	1	1	4
CS HFD Week 7	30246	7	1	1	5
CS HFD Week 9	31465	5	2	0	3

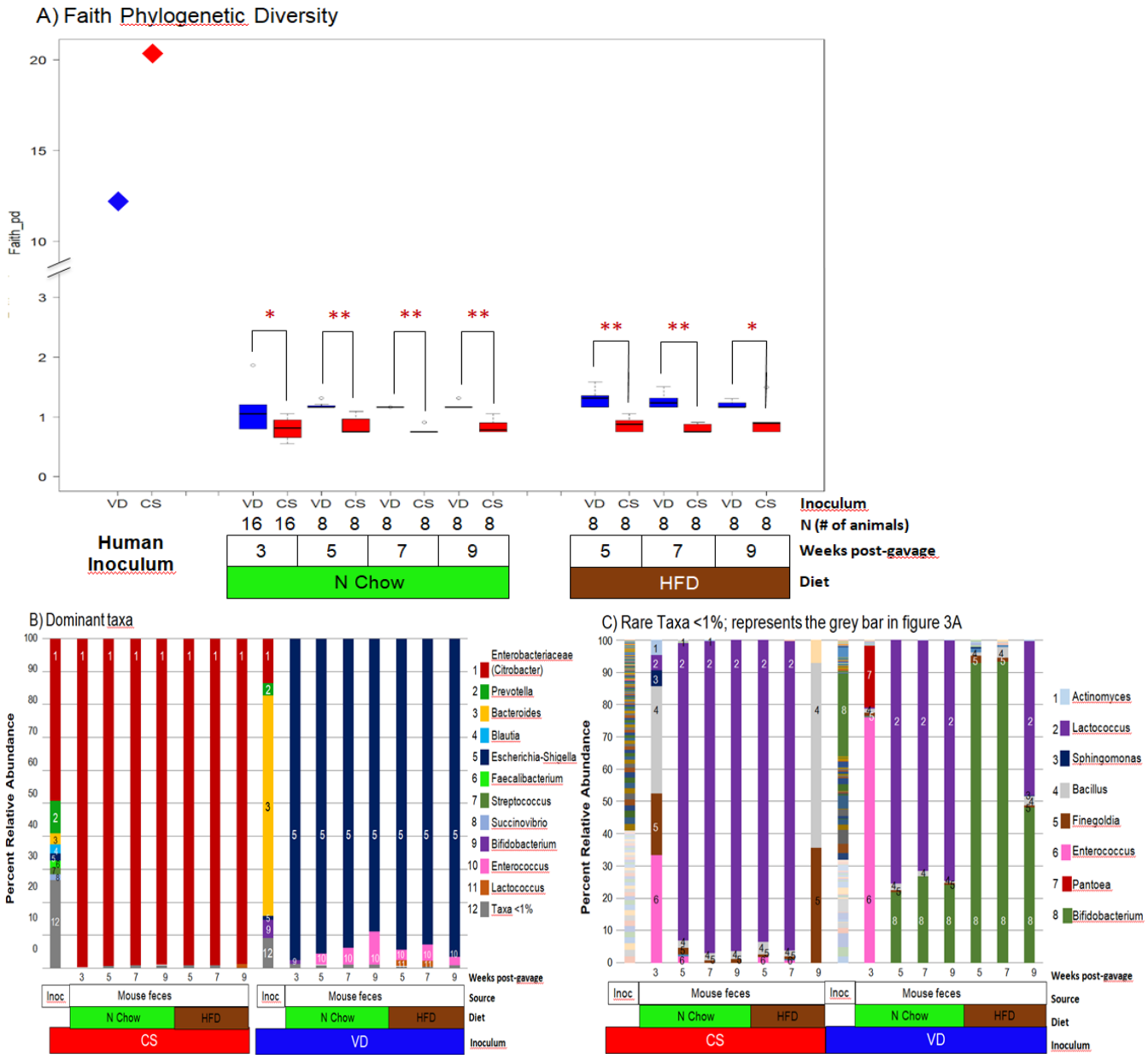

Fig 2. Alpha diversity of the mouse microbiota after humanization of GF mice with neonatal feces and microbiome compositions. A=Faith Phylo Div.; Barplots of dominant taxa ≥1%; C=Rare taxa < 1%. Red shaded areas represent periods of high fat diet.In the human inoculum, diversity was higher in infants born by CS in relation to VD. Diversity that engrafted in the mice was lower than in the infant feces. Mice receiving feces from the CS inoculum showed lower diversity than those receiving VD inoculum and this was unaffected by time and diet. CS inoculum communities were dominated by Citrobacter while VD inoculum was dominated by Bacteroides. Citrobacter engrafted dominantly in CS mice, but Escherichia-Shigella dominated VD mice communities with a notable presence of Enterococcus

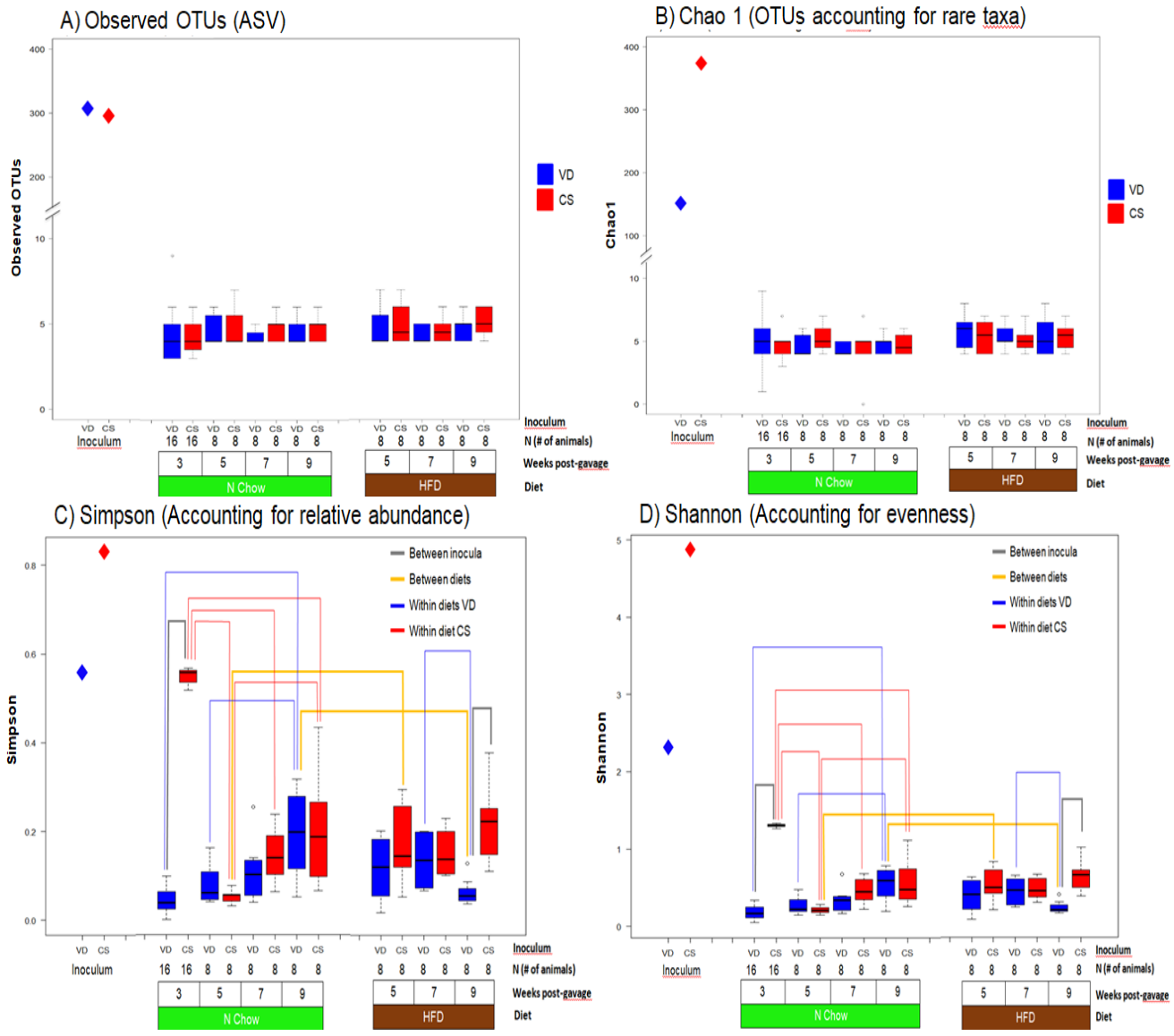

Fig 3. Different metrics of fecal microbiota alpha diversity in the feces of mice conventionalized with human neonate fecal microbiota. A=Observed OTUs. B=Chao1. C=Simpson. D=Shannon. CS Inocula has greater alpha diversity using Chao1, Simpson, and Shannon indices and similar observed OTUs between inocula. Mouse alpha diversity is not significantly different between diet and source of inocula. Alpha diversity increases over time in Simpson and Shannon indices in normal chow samples.

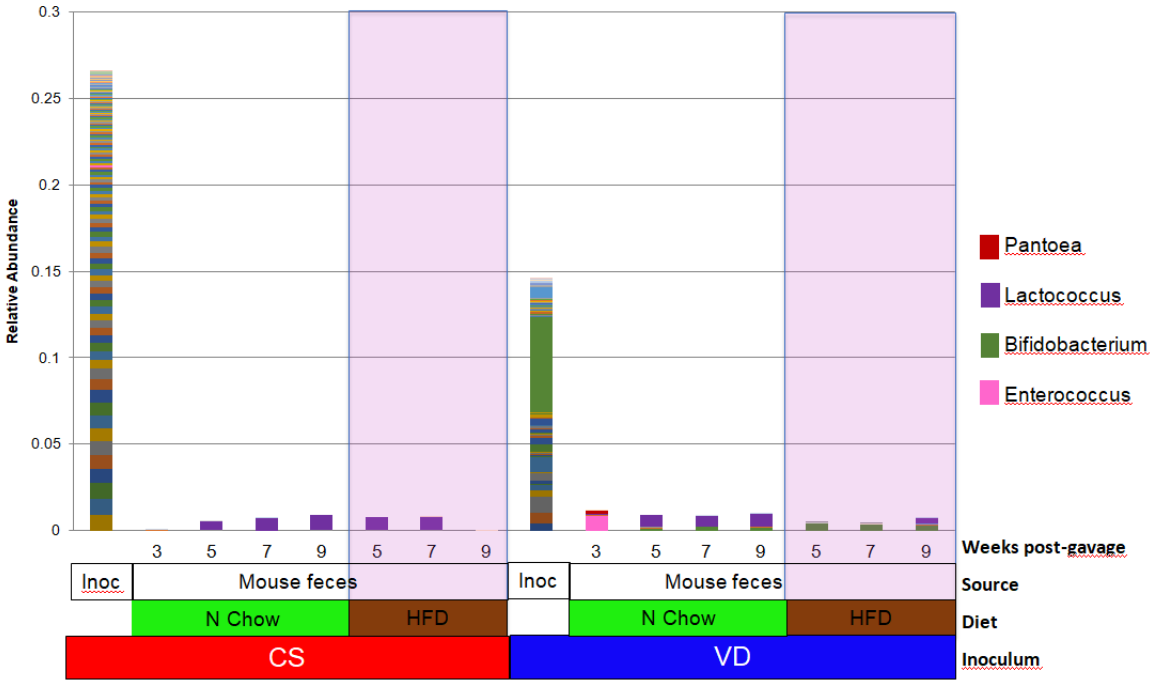

Fig 4. Relative of rare taxa <1% in feces of humanized gf mice differing by birth mode of donor. Represents the grey 1% bar in figure 2B with actual abundances. Pink shaded areas represent times of high fat diet. CS inocula had 173 rare taxa compared to its 8 dominant taxa and comprised 26.6% of the total community. VD inocula had 96 rare taxa compared to its 5 dominant taxa and the rare taxa comprised 14.6% of the community.When engrafted CS mice had 6 rare taxa engrafted to VD mice 15 rare taxa engrafted.

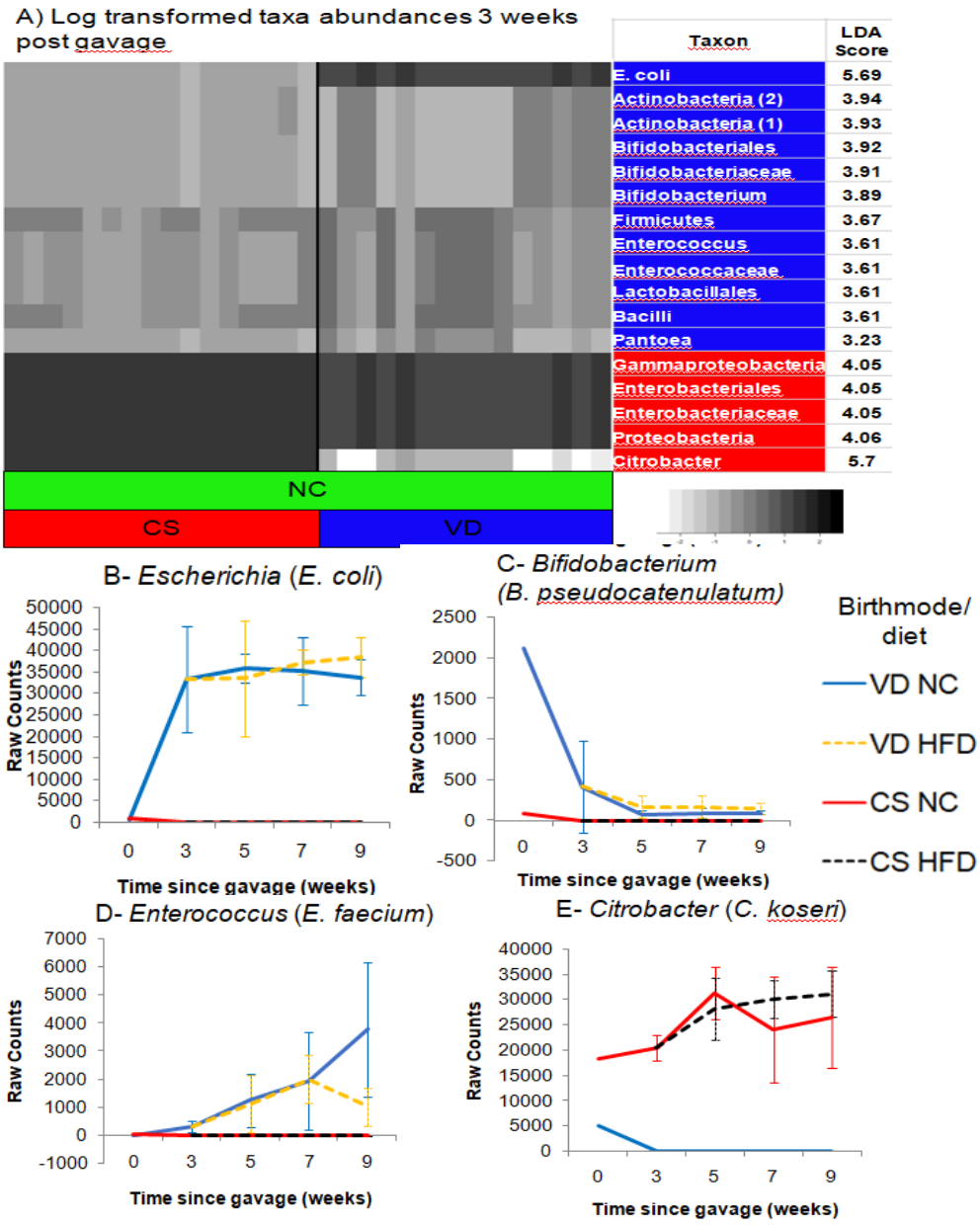

Fig 5. Community compositions of LEfSe discriminant taxa. A=Heatmap of taxa abundances at 3 weeks post gavage in mice consuming normal chow (NC), and gavaged with either CS or VD inoculum. Numbers in parenthesis represent taxonomy level. B=Escherichia C= Enterococcus. D=Bifidobacterium. E=Citrobacter. 0 time represents the abundance in the human inoculum. Taxa in panels B-E are genus level taxa, with 100% identity in Blastn and represent average counts of each mice treatment group, namely birthmode of the human infant feces gavaged into mice and diet of the mice (as shown the legend).Citrobacter koseri solely dominated CS mice communities while E. coli dominated VD mice communities along with the presence of Bifidobacterium, Lactococcus, and Pantoea In raw counts, Citrobacterkoseri solely dominated CS mice with minute amounts in VD mice while E. coli dominated VD mice with no presence in CS mice along with a growing presence of Enterococcus in VD mice and decreasing presence of Bifidobacterium.

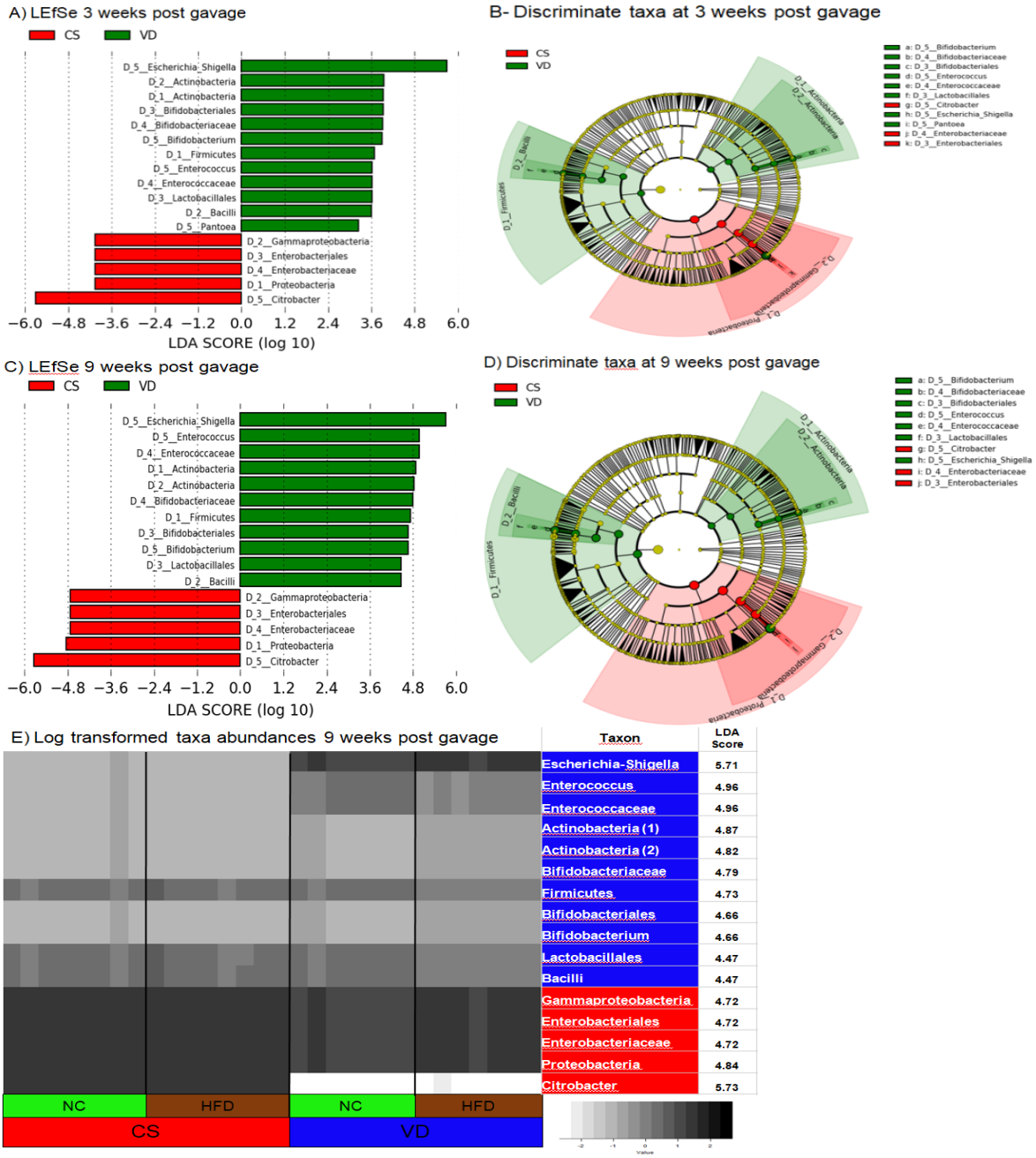

Fig 6. Linear discriminant analysis effect size (LEFSe) barplot of statistically significant taxa engrafted in mice. A=LDA barplot of 3 weeks post gavage; B=Linear discriminant analysis effect size (LEFSe) cladogram of 3 weeks; C=LDA barplot of 9 weeks; D=Linear discriminant analysis effect size (LEFSe) cladogram of 9 weeks; E=Heatmap of LEFSe significant taxa 9 weeks post gavage. Escherichia-Shigella, Bifidobacterium, Enterococcus andPantoea associated with VD inocula while Citrobacter associated with CS inocula. Based on the cladogram, VD inocula was associated with Actinobacteria and Firmicutes while CS inocula was associated with Proteobacteria with the sole exception being Escherichia-Shigella (BLAST as E. coli) being associated with VD inoculated mice.

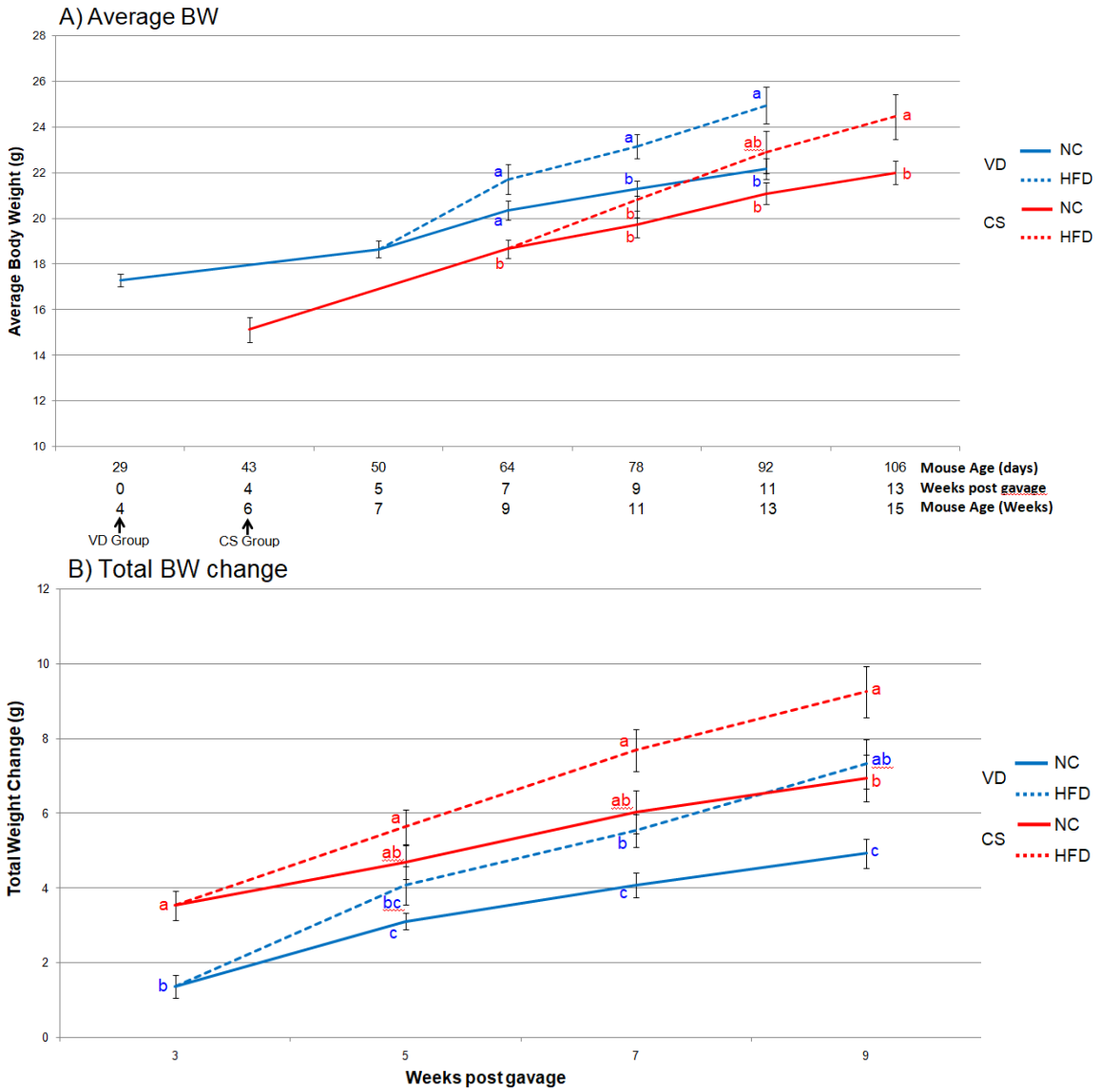

Fig 7. Body weight of humanized GF mice over a 9 week period. A=Average body weight; B=Total body weight change. Original inocula were: feces from babies born by CS (red), born vaginally (VD=blue). HFD was given at Age 50 for VD group and age 64 for CS group and is represented by dotted lines. CS mice were significantly smaller than VD mice when inoculated and were inoculated 2 weeks later than VD mice. Total body weight change for CS mice was significantly greater than VD mice at all time points; however, starting BW and differences in time of inoculation renders the data untrustworthy.

Table 2. Number of classified and unclassified reads at different phylum levels in inocula from babies born vaginally (VD) or by Cesarean (CS), or in conventionalized mice. Classified using the Silva132 taxonomy classifier in QIIME2. There were negligible levels of unclassified taxa.

Group	Taxonomic Level	Domain	Phylum	Class	Order	Family	Genus
CS Inoculum-human	# Reads Classified	37146	37144	37144	37144	37066	18653
	# Reads Unclassified	2	4	4	4	82	18495
	# Taxa Classified	2	17	25	46	76	158
	# Taxa Unclassified	1	2	2	2	8	23
VD Inoculum-human	# Reads Classified	38333	38331	38329	38329	38316	33144
	# Reads Unclassified	0	2	4	4	17	5189
	# Taxa Classified	2	12	18	30	50	88
	# Taxa Unclassified	0	1	2	2	3	12
Mouse feces - CS humanized microbiota	# Reads Classified	20359	20539	20539	20539	20359	21
	# Reads Unclassified	0	0	0	0	0	20338
	# Taxa Classified	2	3	5	6	7	6
	# Taxa Unclassified	0	0	0	0	0	1
Mouse feces - VD humanized microbiota	# Reads Classified	34114	34114	34114	34114	34114	34113
	# Reads Unclassified	0	0	0	0	0	1
	# Taxa Classified	2	6	8	15	16	16
	# Taxa Unclassified	0	0	0	0	0	1

DISCUSSION

Differences in human microbiota of newborns by delivery mode

Birth mode had a significant impact on the composition of human fecal microbiota from infants and the diversity observed. Infants born by CS have lower Bifidobacterium and Bacteroides (Dominguez-Bello et al., 2010, Reyman et al., 2019b, Rutayisire et al., 2016, Shao et al., 2019, Yassour et al., 2016, Jakobsson et al., 2014, Bokulich et al., 2016, Yang et al., 2019). In addition, Enterobacteriaceae (Rutayisire et al., 2016)and Parabacteroides and Clostridium could also be lower in CS babies compared to VD(Mueller et al., 2017).

In the present study, the pooled fecal inoculum from C-section born neonates showed higher bacterial alpha diversity at 2 days of age in relation to those born vaginally. Although differences in alpha diversity have been reported non-significant (Reyman et al., 2019b), other studies report that babies born by CS have an initial higher alpha diversity (Wong et al., 2020) and a later lower alpha diversity as compared to VD born (Bokulich et al., 2016, Jakobsson et al., 2014, Yang et al., 2019, Yassour et al., 2016).

Beta diversity differences are clear by delivery mode, particularly after the first weeks(Shao et al., 2019, Wong et al., 2020), with smaller differences at birth (in the meconium) (Bokulich et al., 2016, Dominguez-Bello et al., 2010, Mueller et al., 2017)

In addition, CS leads to reduced Th1 responses (Jakobsson et al., 2014) consistent with the association in humans with immune disorders (Kristensen & Henriksen, 2016, Sevelsted et al., 2014).

Fecal transfer

Fecal microbiota transplant (FMT) between human adultsis normally performed in patients with depleted diversity due to infection or other conditions, and has proven efficient in curing Clostridium difficile infections (Chehri et al., 2018, Di Bella et al., 2015). It results in increased alpha diversity in recipient similar to donor(Staley et al., 2017b, Staley et al., 2019, Huang et al., 2019, Vaughn et al., 2016), with similar composition to that in the donor (Staley et al., 2019, Shahinas et al., 2012, Staley et al., 2017b, Ohara, 2019, Jacob et al., 2017)

Recently, a paper published restoration of the microbiota of Cesarean born babies by FMT from their mothers (Helve et al., 2019, Korpela et al.). The microbial communities after FMT in CS babies resembled more those of VD babies, at least during the 3 months of the study (Helve et al., 2019, Liu et al., 2017, Korpela et al.). Restoration was precisely of the Bacteroides, Bifidobacterium, and Enterobacteriaceae that are reduced in the CS born babies (Helve et al., 2019, Korpela et al.).

Mice with microbiota perturbations can be normalized by fecal tranplant (Le Bastard et al., 2018, Le Roy et al., 2019, Pebenito et al., 2019, Riquelme et al., 2019), specially if the recipient is young (Ellekilde et al., 2014, Le Roy et al., 2019). Fecal transplant into antibiotic treated mice reduced Proteobacteria in gut communities bringing abundances closer to donor communities (Ojima et al., 2020)

Germ free mice offer a unique model of engraftment and colonization (Le Roy et al., 2019). These mice can be transferred human microbiota, to study functions that might be conserved in mammals, and thus extrapolated to humans. However, as in our study, mice engraft far less diversity than is in the original inoculum which could be due to the age of recipient mice, young adults, receiving feces from neonates. Adult human feces into mice lead to engraftment of lower diversity than the donor microbiota (Pebenito et al., 2019, Riquelme et al., 2019), but in our case, the remarkably low engraftment might be due to the structure of the microbiota in a 2 day old human neonate, whioch is very different to that in adults. In some experiments, alpha diversity in humanized mice grew with time, until not significantly different from donor (Staley et al., 2017a).

As in our study, beta diversity differences remained between the human inoculum and the recipient GF mice (Staley et al., 2017a, Pebenito et al., 2019)

Bacteroideshas been reported to engraft well from human to mice gut(Staley et al., 2017a, Zhou et al., 2019), but other taxa from donors are lost on humanization mice (Staley et al., 2017a, Pebenito et al., 2019, Zhou et al., 2019, Riquelme et al., 2019). In our study few human taxa engrafted into mice, and communities were dominated by single taxa compared to the more diverse described in other studies(Staley et al., 2017a, Turnbaugh et al., 2009)Bacteroides, noted to engraft from human to mice communities, did not engraft at all in our humanized mice.

Effect of diet on the microbiome (HFD)

HFD did not have an effect on the microbiotas structure of the mice in this study, consistent with other studies (Daniel et al., 2014), but contrary to other studies showing decreased alpha diversity in mice (Hildebrandt et al., 2009, Turnbaugh et al., 2008). Probably, the diversity in our mice was already too low to be further reduced by HFD.

Other studies also have shown beta diversity differences introduced by HFD (Daniel et al., 2014, Hildebrandt et al., 2009, Turnbaugh et al., 2009), not replicated in our experiment. In other studies, HFD increased abundance of Bacteroides and decreased Proteobacteria (Daniel et al., 2014, Salonen & de Vos, 2014, Turnbaugh et al., 2009).

Limitations of the current study

Analysis of the microbiome was done at genus level, however this results in pooling all ASVs in the genus together, and this can be misleading since this assumes that all ASVs in a genus behave the same way in response both delivery mode birth and engraftment into mice when that may not be the case. As such, it is possible that differences at the ASV can be overlooked and in the future, ASV-level analysis could be important to identify key species within genus related to birth mode microbiota differences.

The use of 16S rRNA for microbiota community analysis cannot accurately represent bacterium abundances and may result in extra ASVs being seen in the analysis. A limitation of amplifying the 16S rRNA gene is the asumption that the copy number is

similar between different bacteria, which is indeed not true. Species can actually have more than one copy of of the 16S gene which throws off the actual abundances of ASVs in the community, for example, E. coli, which was dominant in VD inoculated mice, has anywhere from 2-7 copies, with an average of 4.2 copies of 16S rRNA genes in any individual (Větrovský & Baldrian, 2013);even trying to normalize with the average copy number is not effective unless there is limited variation from average copy number (Starke et al., 2020, Louca et al., 2018). Additionally, variations of nucleotide sequence of the 16S RNA gene in the same genome can result in multiple different ASVs resulting from the same genome further complicating diversity in data analysis when using 16S rRNA in order to sequence communities. Despite the limitations, the method is useful, is extensively adopted in many microbiota studies, and has the potential to be corrected by copy number computationally (Louca et al., 2018).

Conclusion

In conclusion, this study shows that differences in the fecal microbiota of infants born vaginally or by C-section, is reflected in the different engraftment in GF mice.It also shows that the microbiota of human neonates engrafts poorly into GF mice.

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