# Differences in Gut Microbiome in HIV-Infected versus HIV-exposed, Uninfected Infants

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## RESULTS

### Alpha Diversity

**Table 1a. Characteristics of participants by HIV-1 infection status**

<table>
<thead>
<tr>
<th>Group</th>
<th>Male, n (%)</th>
<th>Female, n (%)</th>
<th>Male, n (%)</th>
<th>Female, n (%)</th>
<th>CD4%, median (SD)</th>
<th>p-value</th>
<th>HIV+ (NBF) vs HIV+ (BF)</th>
<th>HIV+ (NBF) vs HIV+ (BF)</th>
<th>WHO weight-for-age Z score, median (SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEU</td>
<td>10 (52.6)</td>
<td>9 (47.4)</td>
<td>4 (20)</td>
<td>16 (80)</td>
<td>39.9 (7.8)</td>
<td>0.048</td>
<td>22.3 (13.5)</td>
<td>0.032</td>
<td>1.1 (1.33)</td>
<td>0.064</td>
</tr>
<tr>
<td>HIV+ (NBF)</td>
<td>6 (66.7)</td>
<td>3 (33.3)</td>
<td>4 (40)</td>
<td>6 (60)</td>
<td>42.8 (7.7)</td>
<td>0.37</td>
<td>35.6 (14.0)</td>
<td>0.211</td>
<td>0.76 (0.64)</td>
<td>0.307</td>
</tr>
<tr>
<td>HIV+ (BF)</td>
<td>4 (40)</td>
<td>6 (60)</td>
<td>0 (0)</td>
<td>10 (100)</td>
<td>37.3 (7.3)</td>
<td>0.087</td>
<td>29.5 (9.1)</td>
<td>0.059</td>
<td>0.81 (0.89)</td>
<td>0.143</td>
</tr>
</tbody>
</table>

**Table 1b. Characteristics of participants by breastfeeding status**

<table>
<thead>
<tr>
<th>Group</th>
<th>Male, n (%)</th>
<th>Female, n (%)</th>
<th>Male, n (%)</th>
<th>Female, n (%)</th>
<th>CD4%, median (SD)</th>
<th>p-value</th>
<th>NBF vs BF</th>
<th>NBF vs BF</th>
<th>WHO weight-for-age Z score, median (SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>10 (52.6)</td>
<td>9 (47.4)</td>
<td>4 (20)</td>
<td>16 (80)</td>
<td>39.0 (12.1)</td>
<td>0.048</td>
<td>0.06</td>
<td>0.46</td>
<td>1.16 (1.16)</td>
<td>0.144</td>
</tr>
<tr>
<td>HEU</td>
<td>6 (66.7)</td>
<td>3 (33.3)</td>
<td>4 (40)</td>
<td>6 (60)</td>
<td>42.8 (7.7)</td>
<td>0.37</td>
<td>0.153</td>
<td>0.2 (0.76)</td>
<td>0.81 (0.89)</td>
<td>0.182</td>
</tr>
<tr>
<td>HIV+ (NBF)</td>
<td>4 (40)</td>
<td>6 (60)</td>
<td>0 (0)</td>
<td>10 (100)</td>
<td>35.6 (14.6)</td>
<td>0.087</td>
<td>0.19</td>
<td>0.69 (1.38)</td>
<td>1.52 (1.21)</td>
<td>0.405</td>
</tr>
</tbody>
</table>

**Fig 1: a: HEU (green) vs HIV+ (red).** Chao1 and ACE (both take into account the number of species or richness), but not Simpson or Shannon (both take into account abundance of species and richness), show significantly higher α-diversity in HIV+ compared with HEU. b: NBF (green) vs BF (red). All measures show significantly lower α-diversity in BF compared to NBF infants.

### Differential Abundance

**Table 2. Differential abundance analysis.**

**Fig 2a: HEU vs HIV+**

**Fig 2b: NBF vs BF**

**Fig 3. Differentially abundant microbial taxa through phylum, class, order, family and genus for a. HEU (red) vs HIV+ (green),** where taxa enriched in HIV+ infants are shown in red and taxa enriched in HEU+ are shown in green and b. NBF (red) vs BF (green), where taxa enriched in NBF infants are shown in red, and taxa enriched in BF infants are shown in green.


## Background

- Increased inflammation and immune activation are features of HIV-1 infection, for which gut dysbiosis is implicated in adults with HIV.
- Little is known about the gut microbiome in HIV-1-infected (HIV+) infants, who also have persistent inflammation and immune activation.
- Our previous studies show that markers of intestinal integrity (zonulin, intestinal fatty acid binding protein) do not differ between HIV+ and HIV-exposed (HEU) infants at 3 months of age, but HIV+ infants had higher concentrations of cytokines (IFNγ, IL-1β, IL-2, IL-6, IL-8, IL-10, TNFα) compared with HEU infants.
- Using residual stool extracts from a rotavirus vaccine trial in sub-Saharan Africa (IMPAACT P1072), we profiled fecal bacterial populations to define HIV-associated gut dysbiosis in HIV+ and HEU infants at 3 months of age, who were breastfed (BF) and non-breastfed (NBF).

## Methods

- 40 infants (20 HEU, 20 HIV+) on co-trimoxazole prophylaxis were selected from a rotavirus vaccine trial, in whom we previously characterized inflammation, immune activation and intestinal integrity markers.
- Infants were selected based on stool availability and breastfeeding with matched HEU control infants.
- 165 rRNA (V3V4) sequences from stool DNA were assigned organizational taxonomic units (OTU) with QIIME.
- Alpha (Chao1, abundance coverage estimator [ACE], Shannon, Simpson) and beta (Bray-Curtis, Jaccard, unweighted and weighted UniFrac) diversity, and differentially abundant taxa (linear discriminant analysis effect size (LEfSe)) were analyzed.
- Multivariate analysis adjusted for HIV status, breastfeeding and gender (results not shown).
- p<0.05 indicated a statistical significance.

## Acknowledgements

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## Conclusions

- Significant differences between the microbiome of HEU and HIV+ infants from sub-Saharan Africa are observed.
- Although multivariate analysis did not show an impact of breastfeeding and gender, effect changes may not be evident due to the small sample size.