HHS Public Access

Author manuscript

Sci Transl Med. Author manuscript; available in PMC 2017 February 10.

Published in final edited form as:

Sci Transl Med. 2016 July 27; 8(349): 349ra100. doi:10.1126/scitranslmed.aaf5103.

Maternal HIV Infection Influences the Microbiome of HIV Uninfected Infants

Jeffrey M. Bender[1,2], **Fan Li**[1], **Shoria Martelly**[3], **Erin Byrt**[4,5], **Vanessa Rouzier**[3], **Marguerithe Leo**[3], **Nicole Tobin**[1], **Pia S. Pannaraj**[1,2], **Helty Adisetiyo**[1], **Adrienne Rollie**[1], **Chintda Santiskulvong**[1], **Shuang Wang**[4], **Chloe Autran**[6], **Lars Bode**[6], **Daniel Fitzgerald**[5], **Louise Kuhn**[4], and **Grace M. Aldrovandi**[*,1,2]

[1]Children's Hospital Los Angeles, Los Angeles, CA 90027, USA

[2]Department of Pediatrics, University of Southern California, Keck School of Medicine, Los Angeles, CA 90033, USA

[3]GHESKIO Centers, The Haitian Group for the Study of Kaposi's Sarcoma and Opportunistic Infections, Port au Prince, Ouest 15727, Haiti

[4]Departments of Epidemiology and Biostatistics, Columbia University, New York, NY 10032, USA

[5]Center for Global Health, Weill-Cornell Medical College, New York, NY 10065, USA

[6]Department of Pediatrics, University of California, San Diego, La Jolla, CA 92093, USA

Abstract

More than one million HIV-exposed, uninfected infants are born annually to HIV-positive mothers worldwide. This growing population of infants experiences twice the mortality of HIV-unexposed infants. We found that although there were very few differences seen in the microbiomes of mothers with and without HIV infection, maternal HIV infection was associated with changes in the microbiome of HIV-exposed, uninfected infants. Furthermore, we observed that human breast milk oligosaccharides were associated with the bacterial species in the infant microbiome. The disruption of the infant's microbiome associated with maternal HIV infection may contribute to the increased morbidity and mortality of HIV-exposed, uninfected infants.

INTRODUCTION

More than 1 million HIV-exposed, uninfected infants are born annually to HIV-positive mothers worldwide (1). This growing population of infants experience higher morbidity and twice the mortality of HIV-unexposed, uninfected infants in the same community (2–9).

[*]Corresponding Author: gracea@mac.com.

Competing Interests: No competing interests for any of the authors.

Data availability: Sequence Read Archive- SRP067165.

Author Contributions: GA, LK, LB, and DF conceptualized the study. JB, FL, and SW performed formal analysis of the data. SR, EB, VR, and ML enrolled patients and collected samples and clinical data. HA, AR, and CS performed sequencing experiments. CA and LB performed oligosaccharide analysis. FL and SW curated all collected data. JB and FL wrote the original draft of the paper. NT, PP, CA, LB, DF, VR, LK, and GA all were involved in reading and editing the paper. JB and FL created the figures. GA and LK provided overall supervision and funding acquisition.

Furthermore, many of these HIV-exposed, uninfected infants go on to experience immunological derangements that may persist into adolescence (10). With the growing number of young women infected with HIV worldwide and the persistently high HIV seroprevalence among childbearing women in sub-Saharan Africa, HIV-exposed, uninfected infants are rapidly becoming another significant medical casualty in the HIV pandemic.

It remains unclear why HIV-exposed, uninfected infants experience higher morbidity and mortality compared to matched HIV-unexposed, uninfected infant controls. The establishment of a normal microbiome in infants greatly influences the development of a healthy infant metabolism and immunity (11). Many studies have now shown that the infant acquires much of its microbiome from the mother and continues to be conditioned with commensal bacteria found in breast milk (12, 13). Studies have further shown that HIV-infected adults experience dysbiosis in their microbiome (14–17). As HIV preferentially targets CD4+ T cells within the gut lamina propria (18), the immune system is less well equipped to interact with the microbes that make up the intestinal microbiome. Culture-based methods looking at the microbiome of mother and infant pairs have shown some associations with HIV infection (19), but how this dysbiosis is established in the HIV-exposed, uninfected infant is poorly understood.

Breastfeeding conveys health benefits in both HIV-infected and HIV-exposed, uninfected infants (20–24). For this reason, breastfeeding in combination with maternal antiretroviral therapy is the recommended form of feeding for HIV-infected women in resource-limited settings (25). Milk is a complex fluid providing not only nutrition to the infant but also many immune active components. How these contribute to infant health remains poorly defined. Human milk oligosaccharides (HMOs) are the third largest constituent of human milk, but they are not digested by humans (26). HMOs are multifunctional, but their major purpose appears to be as a prebiotic providing nutrition to the infant microbiome (26). This in turn conditions the developing immune system (27, 28). We have previously shown that changes in HMO composition influence postnatal transmission and survival of HIV-exposed, uninfected infants (20). We hypothesized that perturbation of both the mother's microbiome and mother's breast milk HMO composition by HIV infection alters the microbiome in HIV-exposed, uninfected infants and may account for some of the immunological and survival differences seen in HIV-exposed, uninfected infants compared to HIV-unexposed, uninfected infants.

RESULTS

Study demographics

We enrolled 50 mother and infant pairs from Port au Prince, Haiti, evenly split between HIV-positive and HIV-negative mothers (Table 1). HIV-positive mothers had more antibiotic exposure during pregnancy and the post-partum period ($p=0.02$) and higher body mass indices (BMI) compared to HIV-negative mothers ($p=0.023$), but otherwise the groups were generally comparable. All HIV-positive mothers were on antiretroviral therapy at the time of presentation and throughout their pregnancy. These mothers had low levels of plasma HIV RNA (68% undetectable) and had correspondingly high CD4 T cell counts (median 567 cells per cubic millimeter; interquartile range [IQR] 369, 681) suggesting preserved immune

function (Table 2). All infants were breastfed (Table 3). None of the mothers were on antibiotics at the time of the study and none of the infants received antibiotics at any point, including trimethoprim sulfamethoxazole prophylaxis. Samples from six different body sites were collected from each pair [mother: areolar skin, breast milk, and vagina; infant: mouth, skin, and stool]. A single infant was found to be HIV-infected through vertical transmission and the corresponding mother-infant dyad was excluded from all further analyses.

Composition of mother-infant microbiomes

We first looked broadly at the microbiomes at each sample site. DNA sequencing of the variable region 4 of the 16S ribosomal RNA gene characterized the bacterial composition of the samples. A total of 77 million reads were classified into 698 operational taxonomic units (OTUs) using standard approaches (29) and mapped to the Greengenes database at ≥97% identity (30). Principal coordinates analysis (PCoA) and multivariate ANOVA revealed site- and individual-dependent effects on bacterial composition (Fig. 1A). Overall alpha diversity within sample diversity was relatively similar between sample sites (Fig. 1B); only vaginal samples demonstrated significantly lower diversity than did the other five sample sites (Shannon index $p=0.021$). Bacterial composition varied by sample site (Fig. 1C, D). For example, at the family taxonomic level, areolar skin swab samples had more Pseudomonaceae compared to breast milk samples. Vaginal samples and infant stool samples were found to have relatively more abundant Bifidobacteriaceae.

Overall, samples were related based on individual, site, and mother-infant pairs. Samples from an individual were more similar to other sites on the same individual than they were when compared to random individuals ($p<0.005$) (Fig. S1A). Likewise, samples from the same body site were more closely related to each other than to random sites ($p<0.005$) (Fig. S1B). When we looked specifically at mother and infant pairs, we found that these pairs were more closely related to each other than to random pairs ($p<0.005$) (Fig. S1C). Comparison of the relatedness of the various sample sites of the matched mother and infant pairs versus random mother and infant pairs by permutation test (unweighted UniFrac, t-test) demonstrated that breast milk was closely related to all infant sites (Fig. S1D). Infant stool was closely related to all mother sample sites.

Minimal effects of HIV infection are detected on the maternal microbiome

We next examined the effect of HIV infection on the maternal microbiome by comparing HIV-infected to uninfected mothers. Principle coordinates analysis revealed minimal separation between the areolar skin, breast milk, or vaginal samples and no differences between HIV positive and HIV negative mothers when each site was analyzed separately (Fig. 2A). Further, there were no significant differences in bacterial diversity identified (Fig. 2B, Table S1).

Comparing the relative abundance of bacterial taxa, we found differences in microbial composition based on maternal HIV status at all three maternal sample sites in unadjusted analysis (Table S2). However, when we adjusted for multiple comparisons, bacterial compositions at all taxonomic levels were mostly similar between the two maternal groups (Fig. 2C and D). We only found one statistically significant (defined as false discovery rate

p-value <0.1) difference at the class level in vaginal samples where Betaproteobacteria was more abundant in HIV negative mothers (Fig. 2E). Given previous studies identifying differences in the stool microbiome based on HIV status, we were surprised to see so few differences in our cohort of mothers. However, these women were relatively healthy, had very high CD4 T cell counts (median 567 cells/mm3), and stool was not tested. Of note, when we further analyzed our data based on the history of maternal antibiotic use and BMI, no differences were identified (Table S3).

Mother's HIV infection influences the infant microbiome

In contrast to the mostly consistent microbial communities identified in the mothers, the microbiomes of HIV-exposed, uninfected and HIV-unexposed, uninfected infants showed striking differences based on mother's HIV status. Although PCoA demonstrated some separation based on sample site, there was no clear separation between HIV-exposed, uninfected and HIV-unexposed, uninfected infants when each site was analyzed separately (Fig. 3A). We observed lower alpha diversity in the stool of HIV-exposed, uninfected infants compared to HIV-unexposed, uninfected infants (Shannon p=0.018, Observed OTU p=0.005, Chao p=0.017) (Fig. 3B, Table S1). Further stratification of the infant gut bacterial diversity by maternal CD4 T cell count (1–350 vs. >350) (Fig. S2A) demonstrated a trend towards loss of diversity with lower CD4 T cell counts. When we looked at maternal viral load (undetectable vs. detectable), we identified a dose-response relationship with HIV-exposed, uninfected infants born to mothers with detectable viral loads showing the least amount of alpha diversity (Fig. S2B).

Furthermore, the bacterial community taxonomic composition of infant stool was significantly altered based on the mother's HIV status. Looking at relative abundances, we observed many differences using unadjusted p-values at all sites and at every taxonomic level (Table S2). After correction for multiple comparisons using false discovery rate p-value <0.10, 31 significant differences persisted between the two groups (Fig. 3C, D, and E). These changes were seen at all taxonomic levels, but were most obvious at the family level, where HIV-exposed, uninfected infants had more abundant populations of Pseudomonadaceae and Thermaceae in their stool compared to HIV-unexposed, uninfected infants. In contrast, HIV-unexposed, uninfected infant stool had significantly more Prevotellaceae, Desulfovibrionaceae, and Alcaligenaceae. HIV-unexposed, uninfected infants also had significantly more Weeksellaceae in their mouth samples compared to the HIV-exposed, uninfected infants.

We looked at these taxa more closely to make sure they were not biased by other factors. We performed a permutational analysis of variance based on distance matrices (PERMANOVA) to identify contributions to the differences seen in infant stool microbiome composition. We found that maternal HIV status had the only statistically significant influence accounting for approximately 8% of the differences (p=0.004) (Table S3). Age was associated with approximately 15% of the variation but did not reach statistical significance (p=0.09). When these identified specific microbes were analyzed in relation to the child's age-adjusted and sex-adjusted weight and length, no associations were found. Adjustments for a child's anthropometric status did not change maternal HIV status associations. When the analysis

was further run to account for the slight variation in age between infants, Prevotellaceae and Pseudomonadaceae remained significantly different between the two groups.

Further evidence for dysbiosis in HIV exposed, uninfected infants

After identifying the differences outlined above in the microbial communities of infants born to HIV-positive mothers, we looked for further evidence of dysbiosis to better understand its origins. We applied a random forest classification scheme (14) to identify taxa associated with HIV status. We found that at the family level, Prevotellaceae, Alcaligenaceae, Desulfovibrionaceae, and Pseudomonaceae in the infant stool were most predictive of mother's HIV status (Fig. 4A). These are the same taxa identified earlier as significantly different in the stool microbiomes of the infants based on mother's HIV status using the corrected false discovery rate p-values based on relative abundances.

We then applied a Bayesian approach (31) to estimate the proportion of microbes in the infant samples that came from the mother. The mouth and skin microbiomes of infants were represented similarly in both the HIV-unexposed, uninfected and HIV-exposed, uninfected infant groups with strong influences from breast milk and areolar skin sites (Fig. 4B). However, among HIV-exposed, uninfected infants, it appeared that a greater proportion of the infant stool traced back to mother's breast milk and areolar skin, although this finding was not statistically significant. This may suggest that direct seeding of the infant gut via the mother's microbiome was increased in the context of HIV exposure.

We further looked at the infant microbial gut community by examining the relative maturity of the stool microbiome. To do this, we applied a previously published model using a random forest regression algorithm to compare our infants against a cohort of healthy Bangladeshi infants (32). A relative maturity index was calculated for each experimental sample based on the relative stool microbiome age compared to the age of the subject at collection. This was then plotted along with the normalized midline of the previously published reference cohort of healthy controls (Fig. 4C). HIV-exposed, uninfected infants had relatively less mature gut microbial communities compared to HIV-unexposed, uninfected infants (Wilcoxon rank sum p value=0.03). When we specifically looked at the 33 age discriminatory taxa, we found that a single species (*Bacteroides fragilis*) was more abundant in HIV-unexposed, uninfected infants (Wilcoxon rank sum p value=0.025) (Table S4).

Our next step was to look at the predicted metagenomic functional content of the studied bacterial communities using PICRUSt analysis (33). Mother's HIV status led to significant differences in infant stool and skin microbial pathways (Table S5). There were no significant differences in any of the mother's sample sites or the infant's mouth based on mother's HIV status. Glycan biosynthesis and metabolism of cofactors/vitamins were both up-regulated in the HIV-unexposed, uninfected infants, but not in HIV-exposed infants. In total, the results of our random forest analysis, source tracker evaluation, relative maturity index calculations, and PICRUSt analyses provided further evidence that HIV infection in mothers was associated with robust changes in the HIV-exposed, uninfected infant microbiome.

Disruptions in human breast milk oligosaccharide (HMO) composition correlate with the infant microbiome

We asked why the HIV-exposed, uninfected infants in our study had significantly different and potentially dysbiotic gut bacterial communities compared to the unexposed infants. We observed that the bacterial communities of mothers with and without HIV infection in our cohort were relatively similar. The disruptions seen in their infants were thus not completely explained by maternal to infant transfer. The normal succession of the infant microbiome is further driven by exposure to food and the environment. All of the infants in our study were breastfed. Given this, we next examined the breast milk from our cohort of mothers in more detail. Here we saw differences between HIV-infected and uninfected mothers in their milk oligosaccharide composition. HMOs are not digestible by infants, but are primarily thought to provide metabolic substrate for specific commensal bacterial communities. Changes in the HMO content of mother's milk may thus have profound downstream effects on the establishment of the infant microbiome.

We used high performance liquid chromatography to characterize the milk oligosaccharides associated with our study samples. The HMO composition of breast milk was altered in HIV-positive compared to HIV-negative women (Fig. 5A). The small sample size meant that these differences did not reach statistical significance; however, they were consistent with differences our group has previously reported from larger samples of HIV-positive and HIV-negative women in Zambia (20, 23). The relative abundance of 3′-sialyllactose (3′SL) (Wilcoxon Rank Sum p=0.067), 3-fucosyllactose (3FL) (p=0.10), and 2′-fucosyllactose (2′FL) (p=0.077) trended towards being increased in breast milk from HIV-positive mothers compared to HIV-negative mothers. In contrast, human milk lacto-N-tetraose (LNT) (p=0.064) and lacto-N-neotetraose (LNnT) (p=0.080) trended towards being increased in HIV-negative mothers. All other HMO comparisons, including the total amount of HMO, total fucosylated, and total sialylated were similar when compared between HIV-positive and HIV-negative mothers.

Surprisingly, we saw few distinct differences in the breast milk bacterial communities between HIV-positive and HIV-negative mothers (Fig. 2). However, when we used Pearson correlations to define the associations between specific HMOs and bacteria, we did see significant direct associations with the infant stool bacteria (Fig. 5B). These correlations were distinct and dependent on mother's HIV status. For example, in HIV-negative mothers, increases in 3′-sialyllactose were correlated with relative increases in Enterococcacae and Fusobacteriaceae in the infant's stool. In contrast, when we looked at HIV-positive mothers, we found that an increase in lacto-N-fucopentaose 1 (LNFP1) in breast milk led to an increase in Bifidobacteriaceae in the infant's stool.

Next, we compared the infant's stool relative maturity index to the HMO composition of mother's breast milk using Spearman correlation (Figure 5C). Lacto-N-tetraose, which was relatively increased in HIV-negative mothers, was also significantly associated with increased microbial maturity in the infant's stool (fdr_p=0.003). In contrast, 3-fucosyllactose was relatively increased in HIV-positive mothers' breast milk and was significantly associated with decreased infant stool microbial maturity (fdr_p=0.058). Taken together, these data demonstrate that the normal HMO composition of breast milk and the correlations

between HMOs and the infant microbiome are lost or disrupted based on maternal HIV infection.

DISCUSSION

In 2013, an estimated 1.5 million women living with HIV gave birth, accounting for nearly 20% of all births in Sub-Saharan Africa (1). Even though mother-to-child transmission rates are decreasing due to improved availability of antiretrovirals (1), there is now a large and rapidly growing cohort of HIV-exposed, uninfected infants. These children experience nearly twice the mortality rates compared to matched controls (7). The results of our study show that maternal HIV infection is associated with changes in the microbiome of HIV-exposed, uninfected infants. Furthermore, the human milk oligosaccharide content trended towards being different based on maternal HIV status. Specific oligosaccharides appeared to be directly linked with specific bacteria within the infant's microbiome. These data suggest that maternal HIV infection disrupts the normal development of the infant microbiome. This relatively immature and dysbiotic microbiome potentially could compromise development of the infant's immune system. This may help to explain the higher morbidity and mortality of HIV-exposed, uninfected infants compared to HIV-unexposed infants in the same communities.

We identified differences in the microbiomes of our cohort of Haitian mother and infant pairs based on mother's HIV status. Most importantly, we found significant differences in the microbiomes of HIV-exposed, uninfected infants compared to the HIV-unexposed, uninfected infants. This was most clearly demonstrated in the stool composition of HIV-exposed, uninfected infants, but was also observed in mouth and skin samples as well. *Prevotella* and *Pseudomonas* species have both been associated with HIV infection in prior microbiome studies (14, 15, 17, 34) and were significantly different in our HIV-exposed, uninfected infant cohort as well. The reason for this relationship in HIV infection remains unclear. *Prevotella*-rich bacterial communities have previously been associated with pro-inflammatory states including bacterial vaginosis associated with HIV transmission (35–37). The gut mucosa of HIV-infected individuals is inflamed. Recent studies suggest that the chronic gut inflammatory states seen in HIV-positive individuals may be related to dendritic cell activation and association with *Prevotella* species (17, 38).

Although prior studies have shown differences in the stool microbiomes of infected versus uninfected individuals (14, 15, 17), the microbiomes in the body sites we investigated (vagina, breast milk, areolar skin) were relatively similar in HIV-infected versus HIV-uninfected women. Initial studies in macaques looking at the stool microbiomes based on the presence or absence of simian immunodeficiency virus (SIV) infection did not show significant differences in the microbial communities between the two groups (39). Similarity in the microbiomes of HIV-uninfected and HIV-infected women in our study is likely explained by the fact that the HIV-infected women in our study had relatively intact immune systems, were virologically suppressed with antiretroviral drug therapy, were well-nourished, and remained relatively healthy on antiretroviral therapy with good follow up and healthcare. We also did not study the mother's stool microbiome; previous studies

examining the microbiomes of HIV-infected individuals have identified dysbiosis of the gut microbiome.

How did the HIV-exposed, uninfected infants develop such clearly different microbiomes compared to those unexposed infants in the same community? We propose that it may be the combination of the slight perturbations in the mother's microbiome and differences in the HMO composition of the mother's breast milk that may explain these differences. Even though there were only minor differences in the mothers' microbiomes, when we traced back to see the origins of the infants' microbiome taxa, there was a clear influence from the mother. This was especially true in the HIV-positive mothers and their exposed infants. Treatment of HIV-infected mothers with antiretroviral drugs may further contribute to the exposed infant's disrupted microbiome. The reason for differences in the HMO breast milk composition observed remain unclear, but also may be associated with HIV infection itself or the antiretroviral medications.

With only very small differences in the breast milk microbial communities, we believe that the HMOs produced in breast milk are not directed toward the growth of specific bacteria within breast milk itself. Rather, we propose that the changes in the HMO composition may have downstream prebiotic effects on the growth and colonization of various bacterial species in the infant microbiome. In this way, HMOs in breast milk may be responsible for the dysbiosis seen in HIV-exposed uninfected infants. We identified clear relationships between specific HMOs altered by HIV infection that were associated with differences seen in the infant's stool microbiome. Many of these same HMOs have been identified as key components of breast milk in prior HIV studies (20, 23). Unfortunately, little has been done to understand the relationships between HMOs and the infant microbiome in humans. Correlations linking mother's breast milk HMOs to specific bacteria have recently been identified in a study of 14 mother and premature infant dyads (40). Furthermore, links between specific prebiotics and *Prevotella* communities in the gut microbiome have been established previously in mice (41). It has also been shown that *Bifidobacterium* species, thought to be beneficial, preferentially predominate in the presence of lacto-N-neotetraose in germ free mice (42).

Identifying these differences in the microbiome of HIV-exposed, uninfected infants and their origins is just the beginning of our understanding of this complex system. We found that the relative microbial maturity of the HIV-exposed, uninfected infants' stool was significantly lower than that observed in the infants born to HIV-negative women. This immaturity of the infant microbiome has been associated with a decreased nutritional state (32). The next step will be to understand the mechanisms by which the microbiome and HMOs work together to impact the health of these at-risk HIV-exposed, uninfected infants. This knowledge can then be applied towards developing interventions to prevent the increased morbidity and mortality seen in HIV-exposed, uninfected infants. Providing infants with important beneficial bacteria (probiotics) or potentially specific milk oligosaccharides (prebiotics) may potentially improve long-term outcomes although this remains to be thoroughly investigated and tested.

In depth studies looking at the impact of prebiotics and probiotics in HIV-exposed, uninfected infants are needed. Already, studies in macaques have shown modest benefit in

preventing long-term gastrointestinal complications with the administration of probiotics in combination with prebiotics (43). A study looking at treating children in Malawi with high HIV prevalence for severe acute gastroenteritis, did not show improvement in outcomes with the use of combined prebiotic and probiotic therapy (44). Probiotics alone appear to be useful in decreasing the severity of necrotizing enterocolitis in premature HIV-exposed, uninfected infants, but do not work as well as they did in the HIV-unexposed, uninfected cohort (45). With better understanding, we may be able to target bacteria and HMOs that are most needed for normal development and protection against morbidity and mortality in HIV-exposed, uninfected infants.

Our study has several limitations. We looked at a cross-sectional sampling of an at-risk population at a single site. The women in our study had relatively high CD4 T cell counts and we have previously shown that infant morbidity and mortality among HIV-exposed, uninfected infants is inversely related to maternal CD4 T cell count (8). Prospective, long-term, multi-center enrollment may better identify differences in microbiomes and maternal breast milk HMO composition over time. That being said, this is a relatively large microbiome study cohort with 50 mother and infant pairs including multiple sample sites. Furthermore, many of the findings, such as some of the differences seen in bacterial taxa and HMOs, have been identified in previous HIV studies from different geographic locations. HIV-infected mothers in our cohort had higher body mass indices and received more antibiotics during pregnancy or the peripartum period than did HIV-uninfected mothers. However, these parameters did not influence the microbiome of the infant and adjustment for these and other covariates did not change associations of the infant microbiome with maternal HIV status. Selection bias in this impoverished community may have selected a cohort of HIV-negative mothers at higher risk of microbiome dysbiosis. This might explain why clear differences were not observed in the microbiomes of HIV-infected and uninfected mothers. Nevertheless, this bias is towards the null suggesting that the differences between HIV-exposed and HIV-unexposed infants that we observed may be more marked than reported here. We found that maternal HIV infection was associated with changes in the microbiome of HIV-exposed, uninfected infants and further observed that human breast milk oligosaccharides were associated with the bacterial species in these high-risk infants. This disruption of the infant's microbiome may contribute to the increased morbidity and mortality of HIV-exposed, uninfected infants.

MATERIALS and METHODS

Study Design

We conducted a cross-sectional study of 50 mother and infant pairs attending The Haitian Group for the Study of Kaposi's Sarcoma and Opportunistic Infections (GHESKIO) clinics in Port au Prince, Haiti: twenty-five women were HIV-infected and 25 were HIV-uninfected. Women over 15 years of age who had delivered a baby in the past 6 months and who were breastfeeding were eligible to participate in this study. Participants were excluded if they were unable to provide informed consent or if their child did not primarily feed via the breast. This study was approved by the Institutional Review Boards of Children's Hospital Los Angeles, Columbia University, and GHESKIO.

At the time of the study visits, mothers were interviewed by study personnel to collect clinical data. This included sociodemographic data, current medications, pregnancy and delivery history, and infant feeding details. All clinical data were entered into a database on site.

Sample Collection

We prospectively collected demographic and clinical data and six samples from each pair (mother: areolar skin, breast milk, and vagina; infant: mouth, skin, and stool). Stool and breast milk samples were collected in sterile containers and frozen at −20 degrees Celsius. Areolar skin, vaginal, skin, and mouth mucosa swabs were placed in PSP DNA stabilization buffer (Stratec) prior to being frozen. A separate control swab was exposed to the air in the room at the time of collection and was placed in the buffer as well.

Extraction

DNA was extracted from all sample types using the NucliSENS easyMag per manufacture's protocol. We homogenized the frozen stool and breast milk samples in PSP stool stabilization buffer first. Then we used the PSP Stool Spin kit for extraction following the manufacture's protocol, substituting Lysing Matrix E tubes (MP Biomedicals) for the provided zirconia beads. Extracted DNA was stored in elution buffer at −80 degrees Celsius.

Library Preparation

We conducted library preparation on the extracted DNA from the 6 samples above for each mother infant pair. The 16S rDNA was amplified in triplicate and barcoded using a previously published protocol (46). Briefly, this protocol utilizes the V4 region of the 16S rRNA gene. Illumina flow cell adapter sequences and a twelve base pair barcode region were incorporated into the PCR primers. DNA amplicon concentrations were then quantified on a 2200 TapeStation (Agilent Technologies).

Sequencing

We followed the detailed massively parallel sequencing protocol as presented previously by Caporaso, et al(46). Briefly, we pooled the amplicons and diluted to 2nM. The amplicons were then denatured and loaded on a MiSeq desktop sequencer (Illumina) using 2×150bp v2 chemistry. After cluster formation, the amplicons were sequenced with specific primers designed to be complimentary to the V4 amplification primers. The barcode was read using a third sequencing primer in an additional cycle.

HMO Analysis

High-performance liquid chromatography (HPLC) was used to characterize HMOs in breast milk as previously described (23). Briefly, 20 uL of human milk were spiked with the non-HMO raffinose as an internal standard to allow for absolute quantification. Oligosaccharides were extracted by high-throughput solid phase extraction over C18 and Carbograph microcolumns and fluorescently labeled with 2-aminobenzamide (2AB). Labeled oligosaccharides were analyzed by HPLC on an amide-80 column (4.6 mm inner diameter 3 25 cm, 5 mm; Tosoh Bioscience) with a 50-mmol/L ammonium formate–acetonitrile buffer

system. Separation was performed at 25C and monitored with a fluorescence detector at 360 nm excitation and 425 nm emission. Peak annotation was based on standard retention times and mass spectrometric analysis on a Thermo LCQ Duo Ion trap mass spectrometer equipped with a Nano-ESI-source. The total concentration of HMOs was calculated as the sum of the most common oligosaccharides. The proportion of each HMO per total HMO concentration was calculated.

Statistics

Analysis of the sequencing data was carried out using QIIME 1.8.0 (29). Briefly, reads were merged using fastq-join and then demultiplexed. Open reference OTU picking was performed using UCLUST at 97% similarity using the Greengenes 13.8 database (30, 47). Chimeras were removed using the Mothur implementation of ChimeraSlayer (48). The resulting OTU table was filtered at a minimum abundance threshold of 0.005%, giving a total of 698 OTUs. A rarefaction depth of 16,737 reads was selected as most samples were sufficiently saturated. Alpha and beta diversity statistics and taxonomic compositions were calculated as previously described (29).

Additional statistical analyses were performed using R (version 3.0.3). For comparisons of demographic data, either Fisher's exact test or Wilcoxon rank-sum tests were used as appropriate. Comparisons of alpha diversity and beta diversity distances were performed using a nonparametric t-test with 1000 permutations. Comparisons of bacterial abundances were performed using a bootstrapped Kruskal-Wallis test with 1000 permutations. Comparisons of HMO composition were performed using a Wilcoxon rank-sum test. All p-values were adjusted for multiple comparisons using the Benjamini-Hochberg method, except where indicated otherwise. False discovery rate (FDR) corrected p-values of <0.1 were considered significant. We assessed the genetic effects on microbiome composition by comparing distances between true mother-baby pairs versus 1000 permutations of randomly drawn pairs of mothers and infants.

Random forest analysis was performed as previously described (15), using HIV status as the outcome and family-level taxa abundances as the covariates. Source tracking analysis was performed using SourceTracker v0.9.5 (31). Relative maturity distributions between HIV-exposed, uninfected and HIV-unexposed, uninfected infants were compared using a Wilcoxon rank-sum test. Analysis of the predicted metagenome was performed using the PICRUSt software package (33). To help identify truly important relationships, significant differences were accepted only for FDR corrected p-values <0.05 in this metagenome analysis.

To identify relationships between HMO levels and selected bacterial abundances, Spearman correlation coefficients were calculated for all pairs of HMO levels and bacterial abundances where at least 10 samples had non-zero abundance. P-values were calculated using 1 million permutations. As the purpose of this analysis was primarily hypothesis generation, all relationships with a FDR corrected p-value <0.2 were accepted as significant. We further evaluated HMOs compared to the relative maturity index using Spearman correlations. FDR corrected p-values <0.1 were considered significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank The Haitian Group for the Study of Kaposi's Sarcoma and Opportunistic Infections (GHESKIO) Clinic in Port Au Prince, Haiti, for all of their help with this project. Further, we would not be able to do this type of research without access to the University of Southern California Center for High Performance Computing.

Funding Support: Funding support for this project was provided by NIDCR R01 DE 0212380 and UM1AI106716 (IMPAACT LC). JB is further funded through a CHLA K12 HD 052954-09 young investigator award.

References

1. United Nations Programme on AIDS. UNAIDS Gap Report. 2014.
2. von Mollendorf C, von Gottberg A, Tempia S, Meiring S, de Gouveia L, Quan V, Lengana S, Avenant T, du Plessis N, Eley B, Finlayson H, Reubenson G, Moshe M, O'Brien KL, Klugman KP, Whitney CG, Cohen C. Increased Risk for and Mortality From Invasive Pneumococcal Disease in HIV-Exposed but Uninfected Infants Aged <1 Year in South Africa, 2009–2013. Clin Infect Dis. 2015; 60:1346–1356. [PubMed: 25645212]
3. Slogrove A, Reikie B, Naidoo S, De Beer C, Ho K, Cotton M, Bettinger J, Speert D, Esser M, Kollmann T. HIV-exposed uninfected infants are at increased risk for severe infections in the first year of life. J Trop Pediatr. 2012; 58:505–508. [PubMed: 22555385]
4. Chilongozi D, Wang L, Brown L, Taha T, Valentine M, Emel L, Sinkala M, Kafulafula G, Noor RA, Read JS, Brown ER, Goldenberg RL, Hoffman I. Morbidity and mortality among a cohort of human immunodeficiency virus type 1-infected and uninfected pregnant women and their infants from Malawi, Zambia, and Tanzania. Pediatr Infect Dis J. 2008; 27:808–814. [PubMed: 18679152]
5. Taron-Brocard C, Le Chenadec J, Faye A, Dollfus C, Goetghebuer T, Gajdos V, Labaune JM, Perilhou A, Mandelbrot L, Blanche S, Warszawski J. Increased risk of serious bacterial infections due to maternal immunosuppression in HIV-exposed uninfected infants in a European country. Clin Infect Dis. 2014; 59:1332–1345. [PubMed: 25053719]
6. Marinda E, Humphrey JH, Iliff PJ, Mutasa K, Nathoo KJ, Piwoz EG, Moulton LH, Salama P, Ward BJ. Child mortality according to maternal and infant HIV status in Zimbabwe. Pediatr Infect Dis J. 2007; 26:519–526. [PubMed: 17529870]
7. Filteau S. The HIV-exposed, uninfected African child. Trop Med Int Health. 2009; 14:276–287. [PubMed: 19171011]
8. Kuhn L, Kasonde P, Sinkala M, Kankasa C, Semrau K, Scott N, Tsai WY, Vermund SH, Aldrovandi GM, Thea DM. Does severity of HIV disease in HIV-infected mothers affect mortality and morbidity among their uninfected infants? Clin Infect Dis. 2005; 41:1654–1661. [PubMed: 16267740]
9. Kuhn L, Aldrovandi GM, Sinkala M, Kankasa C, Mwiya M, Thea DM. Potential impact of new WHO criteria for antiretroviral treatment for prevention of mother-to- child HIV transmission. Aids. 2010; 24:1374–1377. [PubMed: 20568677]
10. Clerici M, Saresella M, Colombo F, Fossati S, Sala N, Bricalli D, Villa ML, Ferrante P, Dally L, Vigano A. T-lymphocyte maturation abnormalities in uninfected newborns and children with vertical exposure to HIV. Blood. 2000; 96:3866–3871. [PubMed: 11090071]
11. Schanche M, Avershina E, Dotterud C, Oien T, Storro O, Johnsen R, Rudi K. High-Resolution Analyses of Overlap in the Microbiota Between Mothers and Their Children. Current microbiology. 2015; 71:283–290. [PubMed: 26044992]
12. Jost T, Lacroix C, Braegger CP, Rochat F, Chassard C. Vertical mother-neonate transfer of maternal gut bacteria via breastfeeding. Environmental microbiology. 2014; 16:2891–2904. [PubMed: 24033881]

13. Martin V, Maldonado-Barragan A, Moles L, Rodriguez-Banos M, Campo RD, Fernandez L, Rodriguez JM, Jimenez E. Sharing of bacterial strains between breast milk and infant feces. Journal of human lactation: official journal of International Lactation Consultant Association. 2012; 28:36–44. [PubMed: 22267318]

14. Lozupone CA, Li M, Campbell TB, Flores SC, Linderman D, Gebert MJ, Knight R, Fontenot AP, Palmer BE. Alterations in the gut microbiota associated with HIV-1 infection. Cell Host Microbe. 2013; 14:329–339. [PubMed: 24034618]

15. Lozupone CA, Rhodes ME, Neff CP, Fontenot AP, Campbell TB, Palmer BE. HIV-induced alteration in gut microbiota: driving factors, consequences, and effects of antiretroviral therapy. Gut Microbes. 2014; 5:562–570. [PubMed: 25078714]

16. Vujkovic-Cvijin I, Dunham RM, Iwai S, Maher MC, Albright RG, Broadhurst MJ, Hernandez RD, Lederman MM, Huang Y, Somsouk M, Deeks SG, Hunt PW, Lynch SV, McCune JM. Dysbiosis of the gut microbiota is associated with HIV disease progression and tryptophan catabolism. Sci Transl Med. 2013; 5:193ra191.

17. Dillon SM, Lee EJ, Kotter CV, Austin GL, Dong Z, Hecht DK, Gianella S, Siewe B, Smith DM, Landay AL, Robertson CE, Frank DN, Wilson CC. An altered intestinal mucosal microbiome in HIV-1 infection is associated with mucosal and systemic immune activation and endotoxemia. Mucosal Immunol. 2014; 7:983–994. [PubMed: 24399150]

18. Brenchley JM, Schacker TW, Ruff LE, Price DA, Taylor JH, Beilman GJ, Nguyen PL, Khoruts A, Larson M, Haase AT, Douek DC. CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. J Exp Med. 2004; 200:749–759. [PubMed: 15365096]

19. Gonzalez R, Maldonado A, Martin V, Mandomando I, Fumado V, Metzner KJ, Sacoor C, Fernandez L, Macete E, Alonso PL, Rodriguez JM, Menendez C. Breast milk and gut microbiota in African mothers and infants from an area of high HIV prevalence. PLoS One. 2013; 8:e80299. [PubMed: 24303004]

20. Kuhn L, Kim HY, Hsiao L, Nissan C, Kankasa C, Mwiya M, Thea DM, Aldrovandi GM, Bode L. Oligosaccharide composition of breast milk influences survival of uninfected children born to HIV-infected mothers in Lusaka, Zambia. J Nutr. 2015; 145:66–72. [PubMed: 25527660]

21. Arpadi S, Fawzy A, Aldrovandi GM, Kankasa C, Sinkala M, Mwiya M, Thea DM, Kuhn L. Growth faltering due to breastfeeding cessation in uninfected children born to HIV-infected mothers in Zambia. Am J Clin Nutr. 2009; 90:344–353. [PubMed: 19553300]

22. Kuhn L, Aldrovandi GM, Sinkala M, Kankasa C, Semrau K, Mwiya M, Kasonde P, Scott N, Vwalika C, Walter J, Bulterys M, Tsai WY, Thea DM. Effects of early, abrupt weaning on HIV-free survival of children in Zambia. N Engl J Med. 2008; 359:130–141. [PubMed: 18525036]

23. Bode L, Kuhn L, Kim HY, Hsiao L, Nissan C, Sinkala M, Kankasa C, Mwiya M, Thea DM, Aldrovandi GM. Human milk oligosaccharide concentration and risk of postnatal transmission of HIV through breastfeeding. Am J Clin Nutr. 2012; 96:831–839. [PubMed: 22894939]

24. Kuhn L, Sinkala M, Semrau K, Kankasa C, Kasonde P, Mwiya M, Hu CC, Tsai WY, Thea DM, Aldrovandi GM. Elevations in mortality associated with weaning persist into the second year of life among uninfected children born to HIV-infected mothers. Clin Infect Dis. 2010; 50:437–444. [PubMed: 20047479]

25. U. N. P. o. AIDS. Guidelines on HIV and Infant Feeding 2010: Principles and Recommendations for Infant Feeding in the Context of HIV and a Summary of Evidence. World Health Organization; Geneva: 2010.

26. Bode L. Human milk oligosaccharides: every baby needs a sugar mama. Glycobiology. 2012; 22:1147–1162. [PubMed: 22513036]

27. Groer MW, Luciano AA, Dishaw LJ, Ashmeade TL, Miller E, Gilbert JA. Development of the preterm infant gut microbiome: a research priority. Microbiome. 2014; 2:38. [PubMed: 25332768]

28. Tobin NH, Aldrovandi GM. Immunology of pediatric HIV infection. Immunol Rev. 2013; 254:143–169. [PubMed: 23772619]

29. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA,

Widmann J, Yatsunenko T, Zaneveld J, Knight R. QIIME allows analysis of high-throughput community sequencing data. Nat Methods. 2010; 7:335–336. [PubMed: 20383131]

30. McDonald D, Price MN, Goodrich J, Nawrocki EP, DeSantis TZ, Probst A, Andersen GL, Knight R, Hugenholtz P. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. ISME J. 2012; 6:610–618. [PubMed: 22134646]
31. Knights D, Kuczynski J, Charlson ES, Zaneveld J, Mozer MC, Collman RG, Bushman FD, Knight R, Kelley ST. Bayesian community-wide culture-independent microbial source tracking. Nat Methods. 2011; 8:761–763. [PubMed: 21765408]
32. Subramanian S, Huq S, Yatsunenko T, Haque R, Mahfuz M, Alam MA, Benezra A, DeStefano J, Meier MF, Muegge BD, Barratt MJ, VanArendonk LG, Zhang Q, Province MA, Petri WA Jr, Ahmed T, Gordon JI. Persistent gut microbiota immaturity in malnourished Bangladeshi children. Nature. 2014; 510:417–421. [PubMed: 24896187]
33. Langille MG, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepile DE, Vega Thurber RL, Knight R, Beiko RG, Huttenhower C. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat Biotechnol. 2013; 31:814–821. [PubMed: 23975157]
34. Iwai S, Huang D, Fong S, Jarlsberg LG, Worodria W, Yoo S, Cattamanchi A, Davis JL, Kaswabuli S, Segal M, Huang L, Lynch SV. The lung microbiome of Ugandan HIV-infected pneumonia patients is compositionally and functionally distinct from that of San Franciscan patients. PLoS One. 2014; 9:e95726. [PubMed: 24752365]
35. Scher JU, Sczesnak A, Longman RS, Segata N, Ubeda C, Bielski C, Rostron T, Cerundolo V, Pamer EG, Abramson SB, Huttenhower C, Littman DR. Expansion of intestinal Prevotella copri correlates with enhanced susceptibility to arthritis. Elife. 2013; 2:e01202. [PubMed: 24192039]
36. Elinav E, Strowig T, Kau AL, Henao-Mejia J, Thaiss CA, Booth CJ, Peaper DR, Bertin J, Eisenbarth SC, Gordon JI, Flavell RA. NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. Cell. 2011; 145:745–757. [PubMed: 21565393]
37. Zalenskaya IA, Joseph T, Bavarva J, Yousefieh N, Jackson SS, Fashemi T, Yamamoto HS, Settlage R, Fichorova RN, Doncel GF. Gene Expression Profiling of Human Vaginal Cells In Vitro Discriminates Compounds with Pro-Inflammatory and Mucosa-Altering Properties: Novel Biomarkers for Preclinical Testing of HIV Microbicide Candidates. PLoS One. 2015; 10:e0128557. [PubMed: 26052926]
38. Dillon SM, Lee EJ, Kotter CV, Austin GL, Gianella S, Siewe B, Smith DM, Landay AL, McManus MC, Robertson CE, Frank DN, McCarter MD, Wilson CC. Gut dendritic cell activation links an altered colonic microbiome to mucosal and systemic T-cell activation in untreated HIV-1 infection. Mucosal Immunol. 2015
39. McKenna P, Hoffmann C, Minkah N, Aye PP, Lackner A, Liu Z, Lozupone CA, Hamady M, Knight R, Bushman FD. The macaque gut microbiome in health, lentiviral infection, and chronic enterocolitis. PLoS Pathog. 2008; 4:e20. [PubMed: 18248093]
40. Underwood MA, Gaerlan S, De Leoz ML, Dimapasoc L, Kalanetra KM, Lemay DG, German JB, Mills DA, Lebrilla CB. Human Milk Oligosaccharides in Premature Infants: Absorption, Excretion and Influence on the Intestinal Microbiota. Pediatric research. 2015
41. Chaplin A, Parra P, Serra F, Palou A. Conjugated Linoleic Acid Supplementation under a High-Fat Diet Modulates Stomach Protein Expression and Intestinal Microbiota in Adult Mice. PLoS One. 2015; 10:e0125091. [PubMed: 25915857]
42. Marcobal A, Barboza M, Sonnenburg ED, Pudlo N, Martens EC, Desai P, Lebrilla CB, Weimer BC, Mills DA, German JB, Sonnenburg JL. Bacteroides in the infant gut consume milk oligosaccharides via mucus-utilization pathways. Cell Host Microbe. 2011; 10:507–514. [PubMed: 22036470]
43. Klatt NR, Canary LA, Sun X, Vinton CL, Funderburg NT, Morcock DR, Quinones M, Deming CB, Perkins M, Hazuda DJ, Miller MD, Lederman MM, Segre JA, Lifson JD, Haddad EK, Estes JD, Brenchley JM. Probiotic/prebiotic supplementation of antiretrovirals improves gastrointestinal immunity in SIV-infected macaques. J Clin Invest. 2013; 123:903–907. [PubMed: 23321668]
44. Kerac M, Bunn J, Seal A, Thindwa M, Tomkins A, Sadler K, Bahwere P, Collins S. Probiotics and prebiotics for severe acute malnutrition (PRONUT study): a double-blind efficacy randomised controlled trial in Malawi. Lancet. 2009; 374:136–144. [PubMed: 19595348]

45. Van Niekerk E, Nel DG, Blaauw R, Kirsten GF. Probiotics Reduce Necrotizing Enterocolitis Severity in HIV-exposed Premature Infants. J Trop Pediatr. 2015

46. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, Owens SM, Betley J, Fraser L, Bauer M, Gormley N, Gilbert JA, Smith G, Knight R. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 2012; 6:1621–1624. [PubMed: 22402401]

47. Goodrich JK, Di Rienzi SC, Poole AC, Koren O, Walters WA, Caporaso JG, Knight R, Ley RE. Conducting a microbiome study. Cell. 2014; 158:250–262. [PubMed: 25036628]

48. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol. 2009; 75:7537–7541. [PubMed: 19801464]

Overline: HIV

One Sentence Summary

Maternal HIV infection may affect the infant's microbiome and this may contribute to the increased morbidity and mortality of HIV-exposed, uninfected infants.

Accessible Summary

Annually there are more than one million children born to HIV-infected women. Most of these children do not acquire HIV infection but they experience twice the mortality of children born to HIV-negative women. We found that maternal HIV infection was associated with changes in the microbiome of these HIV-exposed, uninfected infants. Furthermore, we observed that human breast milk oligosaccharides were associated with the bacterial species in the infant microbiome. The disruption of the HIV-exposed infant's microbiome may contribute to their increased morbidity and mortality.

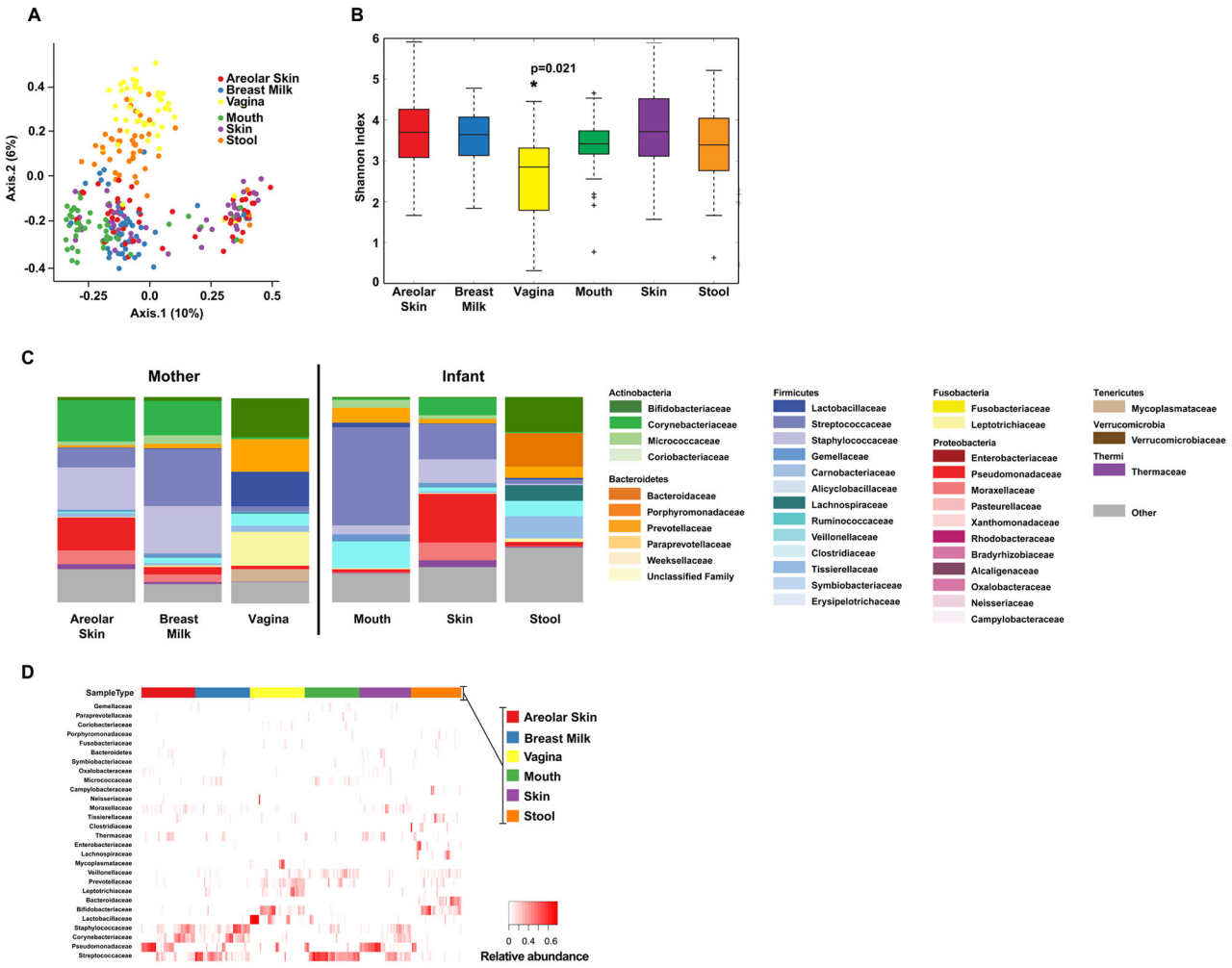

Fig. 1. Microbiomes of 50 mother infant dyads

Overall, we found differences in the microbiomes based on the site of sample collection. (A) Principle coordinates analysis of unweighted UniFrac distances revealed that each sample type clustered into distinct groups. (B) α-diversity (Shannon index) was similar for all sample sites except for the vagina which was significantly less diverse than other sample sites (p=0.021). (C) Bacterial compositions across sample sites (mother: areolar skin, breast milk, vagina; infant: mouth, skin, stool) at the taxonomic level of family. (D) Heatmap representation of individual bacterial compositions at the family level. Families with an overall relative abundance of less than 0.5% are omitted for the sake of clarity.

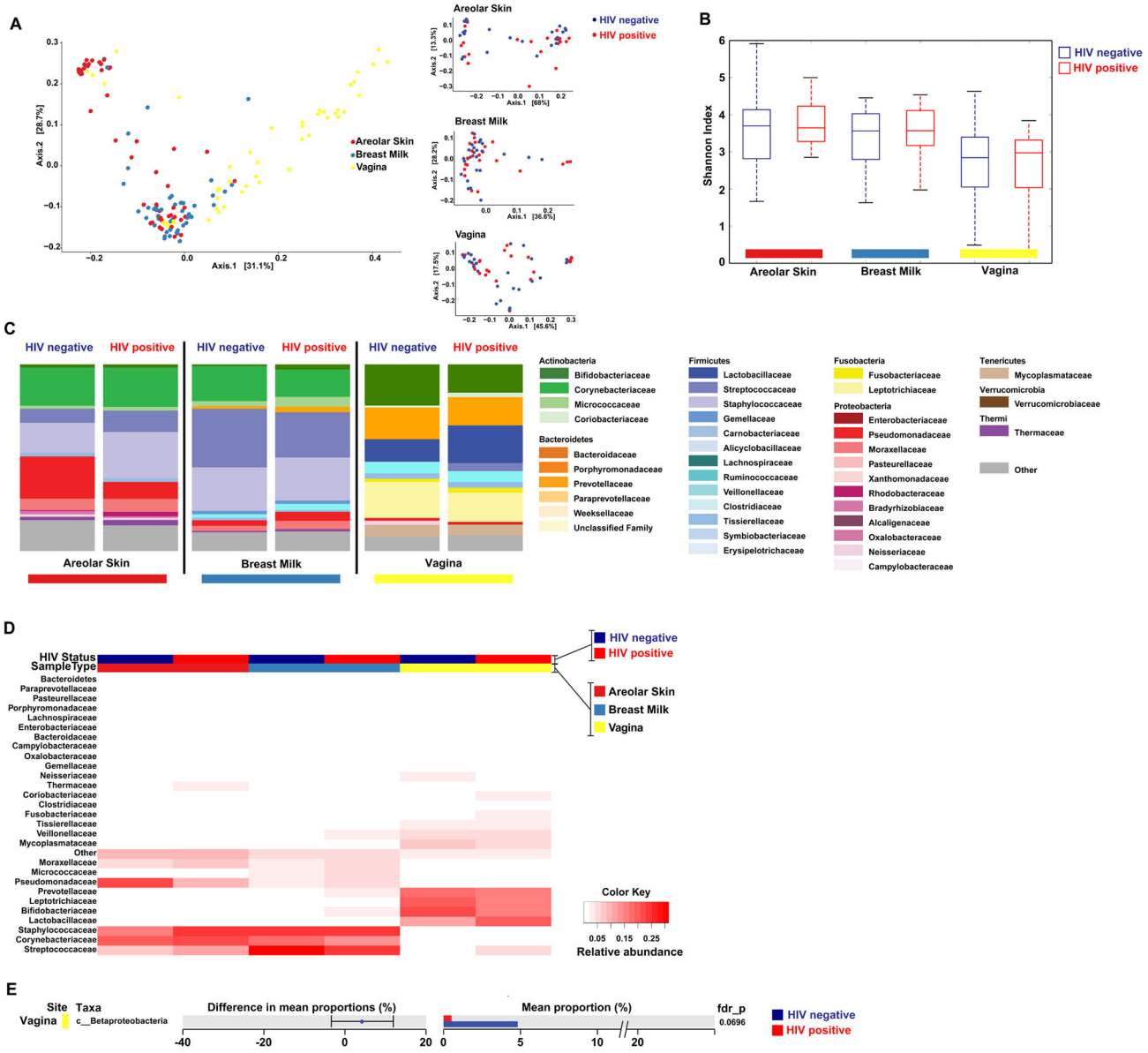

Fig. 2. Minimal effects of HIV infection on maternal microbiomes

(A) Principle coordinates analysis of unweighted UniFrac distances based on mother's HIV status at each sample site (areolar skin, breast milk, vagina). (B) α-diversity (Shannon index) at each sample site based on mother's HIV status. (C) Bacterial compositions across sample sites at the taxonomic level of family. (D) Heatmap representation of individual bacterial compositions at the family level. Families with an overall relative abundance of less than 0.5% are omitted for the sake of clarity. (E) List of all taxa at any level or sample site that were significantly different based on mother's HIV status using corrected p values (fdr_p values <0.1).

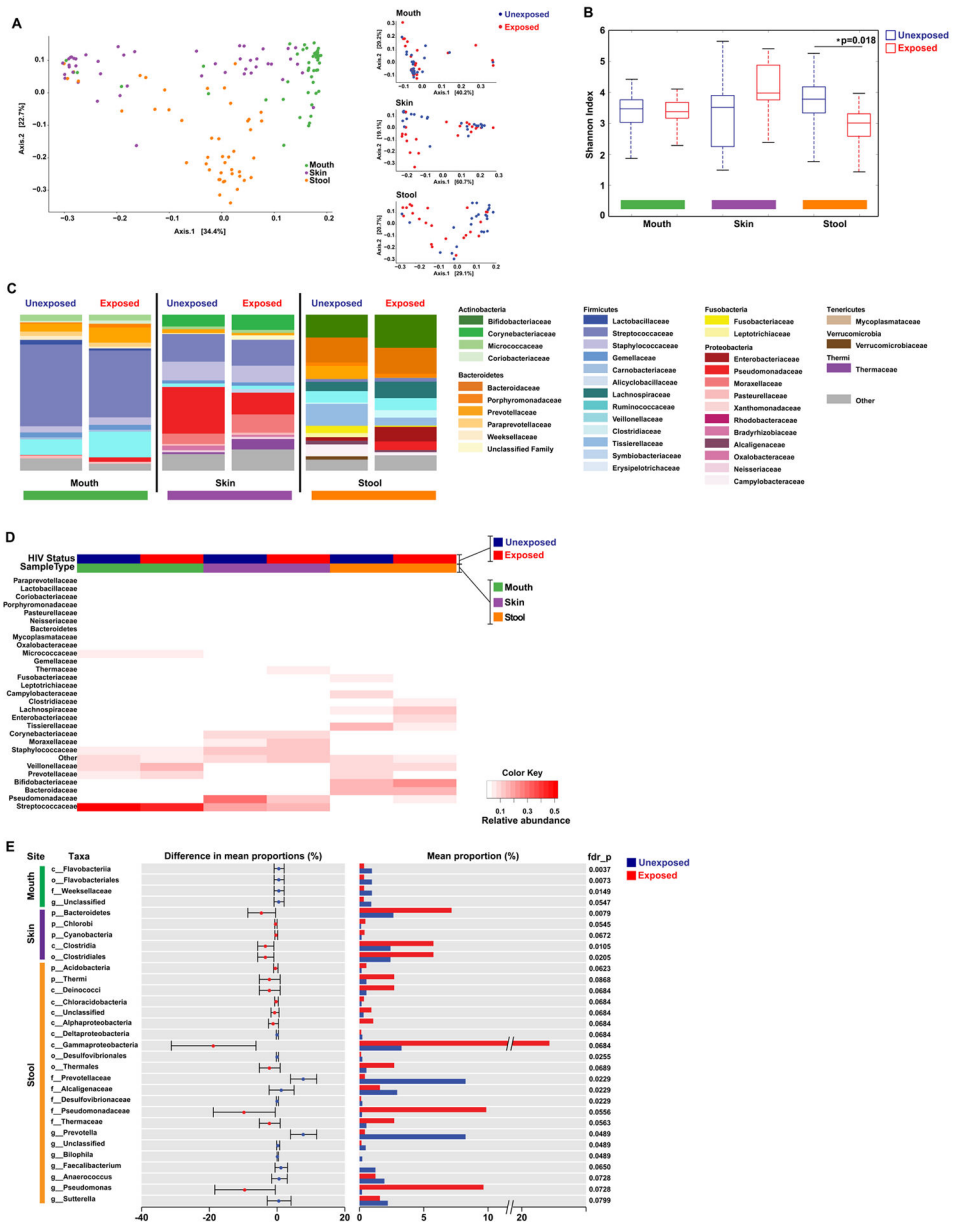

Fig. 3. Effects of maternal HIV infection on the infant microbiome

(A) Principle coordinates analysis of unweighted UniFrac distances based on mother's HIV status at each infant sample site (mouth, skin, stool). (B) α-diversity (Shannon index) at each infant sample site based on mother's HIV status. (C) Bacterial compositions across infant sample sites at the taxonomic level of family. (D) Heatmap representation of individual bacterial compositions at the family level. Families with an overall relative abundance of less than 0.5% are omitted for the sake of clarity. (E) List of all taxa at any family level or infant sample site that were significantly different based on mother's HIV status using corrected p values (fdr_p values <0.1).

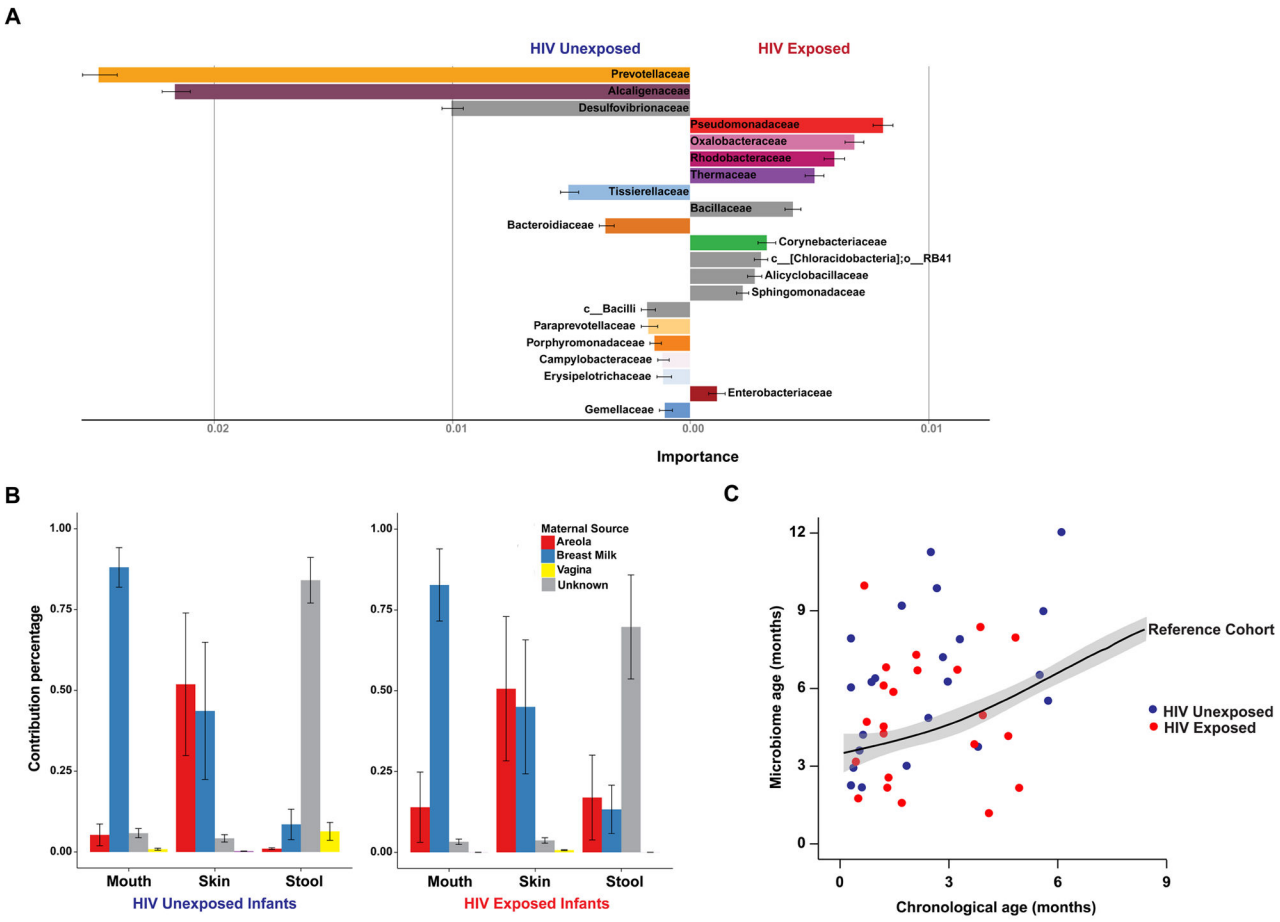

Fig. 4. Microbiome dysbiosis in HIV-exposed, uninfected infants

(A) Random forest classification scheme to identify taxa associated with HIV status. Bacterial taxa enriched in HIV-unexposed infants are represented on the left, with bacterial taxa enriched in HIV-exposed uninfected infants on the right. (B) Bayesian estimate of the proportion of microbes in the infant samples that came from the mother. (C) A relative maturity index was calculated for each experimental sample based on the relative stool microbiome age versus the age of the subject at collection. This was then plotted along with the normalized midline of the previously published reference cohort of healthy controls.

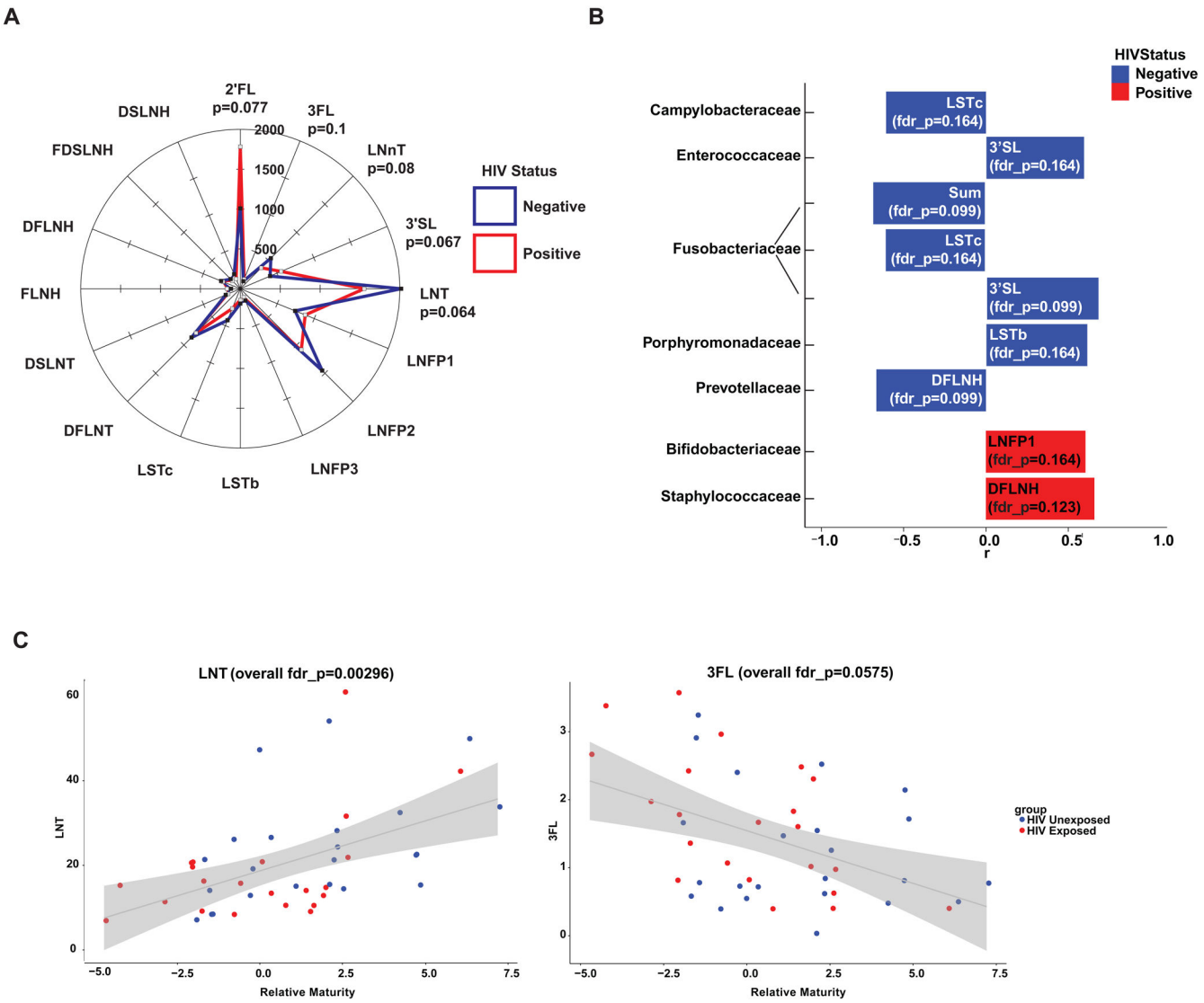

Fig. 5. Human breast milk oligosaccharide (HMO) composition and the infant stool microbiome

(A) The HMO composition of breast milk based on maternal HIV status. Unadjusted p values </=0.1 are shown. (B) Significant associations between specific HMOs and bacterial families. Blue bars represent HIV-unexposed infants. Red bars represent HIV-exposed, uninfected infants. Spearman correlation coefficients are plotted along the x-axis. (C) The infant's stool relative maturity compared to the HMO composition of mother's breast milk using Spearman correlations. The two HMOs with fdr_p values <0.1 are presented: LNT and 3FL. (2′FL= 2′-fucosyllactose; 3FL= 3-fucosyllactose; LNnT= lacto-N-neotetraose; 3′SL= 3′-sialyllactose; LNT= lacto-N-tetraose; LNFP-1= lacto-N- fucopentaose 1; LNFP-2= lacto-N- fucopentaose 2; LNFP-3= lacto-N- fucopentaose 3; LSTb= sialyllacto-N-tetraose b; LSTc= sialyllacto-N-tetraose c; DFLNT= difucosialyllacto-N-tetraose; DSLNT= disialyllacto-N-tetraose; FLNH= fucosyllacto-N-hexaose; DFLNH= difucosyllacto-N-hexaose; FDSLNH= fucosyl-disialyllacto-N-hexaose; DSLNH= disialyllacto-N-hexaose)

Table 1

Demographic and clinical characteristics of 50 mothers from Port Au Prince, Haiti

Half of the mothers were HIV-infected.

Maternal Characteristics	HIV+ mothers (N=25)	HIV− mothers (N=25)	p-value
Maternal Age (years)			
Mean (Standard deviation)	30.2 (5.3)	27.1 (7.5)	
Median (Interquartile range)	31 (28, 34)	27 (20, 31)	0.06
Use of antibiotics during pregnancy or post-partum period (%)	18 (72)	10 (40)	0.02
Vaginal delivery (%)	19 (76)	24 (96)	0.10
Delivery at home (%)	10 (42)	11 (44)	0.87
Term delivery (%)	23 (92)	22 (88)	0.64
Body Mass Index (kg/m^2)			
Mean (SD)	24.7 (4.2)	21.9 (2.8)	
Median (IQR)	25.3 (21.1, 27.5)	21.4 (20.3, 23.4)	**0.023**
Maternal Mid-Upper Arm Circumference (cm)			
Mean (SD)	25.6 (5.5)	26.0 (4.0)	
Median (IQR)	27 (23, 29.0)	26 (24, 28)	0.59
Exclusive breastfeeding (%)	20 (80)	19 (76)	0.73
Breast problems: Cracked nipples, clogged ducts, mastitis (%)	10 (40)	9 (36)	0.77

Table 2

Clinical characteristics of 25 HIV-positive mothers from Port Au Prince, Haiti

All 25 mothers were on combination antiretroviral therapy.

Clinical Characteristics of HIV+ Mothers	**N=25**
Viral Load (Copies/mL)	
Median (IQR)	Undetectable (undetectable, 3300)
Viral load (%)	
Undetectable	17 (68)
Detectable	8 (32)
CD4 T cell count (Cells/mm^3)	
Mean (SD)	553 (289)
Median (IQR)	567 (369, 681)
CD4 count <350 (%)	
>350	20 (80)
1–350	5 (20)
On antiretroviral therapy (%)	25 (100)

Table 3

Demographic and clinical characteristics of 50 infants from Port Au Prince, Haiti

Half of the infants were HIV-exposed, born to HIV-positive mothers. None of the infants received courses of antibiotics. Only one infant subsequently acquired HIV infection. This mother: infant dyad was excluded from subsequent analyses.

Infant Characteristics	HIV-exposed (N=25)	HIV-unexposed (N=25)	p-value
Infant Age (days)			
Mean (SD)	66.6 (44.8)	71.4 (56.6)	0.65
Median (IQR)	48 (33, 113)	72 (16, 96)	
Female sex (%)	13 (52)	13 (52)	1.00
Antibiotics (%)	0 (0)	0 (0)	1.00
Weight-for-age z-score			
Mean (SD)	−0.9 (1.2)	−1.0 (1.3)	0.90
Median (IQR)	−1.2 (−1.6, −0.4)	−0.8 (−1.6, 0.1)	
Weight-for-age z-score (%)			
< −1.5	7 (28)	8 (32)	0.76
>= − 1.5	18 (72)	17 (68)	
Length/height-for-age z-score			
Mean (SD)	−1.2 (1.6)	−0.6 (1.7)	0.25
Median (IQR)	−1.4 (−1.8, 0.0)	−0.1 (−1.0, 0.5)	
Length/height-for-age z-score (%)			
< −1.5	12 (48)	5 (20)	**0.037**
>= −1.5	13 (52)	20 (80)	
HIV infection (%)	1 (4)	N/A	