HIV Exposure Affects Gut Virome Richness but Not Diversity Among Kenyan Infants

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Background

- The gut virome is seeded at birth, develops rapidly in the first years of life, and is influenced by behavioral and environmental factors including feeding and exposure to the maternal virome.
- HIV alters the gut microbiota of people living with HIV, which in turn could affect virome development of children who are HIV exposed, uninfected (CHEU) versus HIV-unexposed (CHUU).
- Gut microbiota help shape gut immunity—differences in gut virome development could contribute to the higher observed morbidity/mortality among CHEU than CHUU.

Objective: This is the first study to longitudinally describe and compare the gut virome of CHEU and CHUU in infancy.

Methods

- This analysis was nested in the Linda Kizazi Study, a Nairobi, Kenya-based birth cohort designed to evaluate how mother-to-child viral transmission affects child health and development.

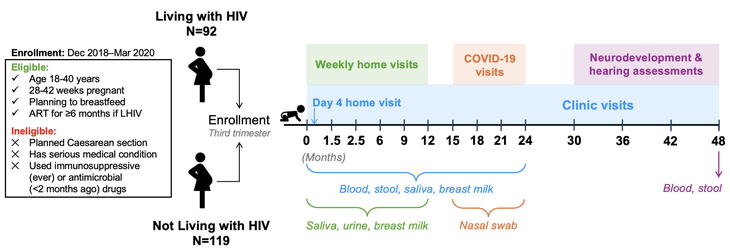


Figure 1. Linda Kizazi Study enrollment and follow-up. Women were recruited from antenatal and HIV care visits at Mathare North Health Centre, a maternal-child health facility serving a densely-populated, low-income area of Nairobi, Kenya. Clinical data collection included current and recent symptoms of illness, diagnoses, medications, immunizations, and infant feeding practices.

- We sequenced the DNA virome in infant stool samples collected between birth and 21 months from 37 CHUU and 32 CHEU whose mothers started ART before or in early pregnancy.
- Virome alpha diversity (richness, Shannon Index) and beta diversity (weighted Bray-Curtis distances) were compared with:
  - Nonparametric statistical tests
  - Generalized estimating equations models adjusted for maternal partnership status and education (alpha diversity)
  - Principal coordinates analysis (PCoA) and a multivariate PERMANOVA (beta diversity)
- Comparison groups were defined by HIV exposure, study visit, and feeding mode (exclusive breastfeeding [BF], mixed feeding, and no BF).
- The MaAsLin2 package in R was used to identify viral taxa with differential relative abundance between CHEU and CHUU.

Results

More CHEU than CHUU were exclusively breastfed for 6 months.

Table. Selected participant characteristics.

Characteristic	N	Overall N = 67†	CHUU N = 36†	CHEU N = 31†
Exclusively breastfed for 6 months	67	47 (70%)	19 (53%)	28 (90%)
Months of any breastfeeding	67	13 (12, 19)	18 (14, 23)	12 (10, 12)
Mother married or steady partner	67	64 (96%)	35 (97%)	29 (94%)
Mother had Secondary Education or More	67	34 (51%)	13 (36%)	21 (68%)

† n (%); Median (Q1, Q3)

All CHEU received ARV and cotrimoxazole prophylaxis. Most (94%) mothers with HIV initiated ART before pregnancy (87% efavirenz-based).

The infant gut DNA virome is dominated by bacteriophages.

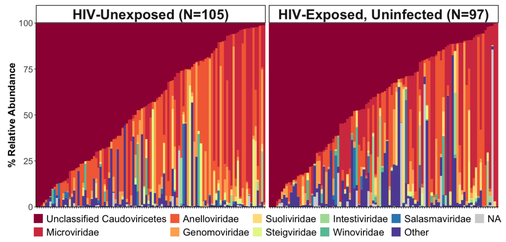


Figure 2. Relative abundance of viral families by HIV exposure.

Beta diversity did not differ by HIV exposure or feeding mode, but varied by age.

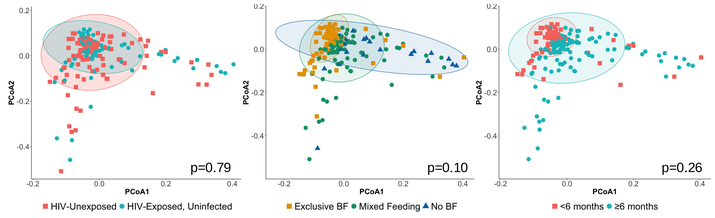


Figure 4. PCoA analysis of weighted Bray-Curtis distances by (A) HIV exposure, (B) feeding mode, and (C) age <6 vs ≥6 months. P-values from PERMANOVA model.

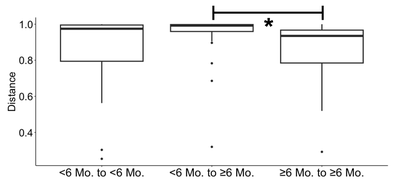


Figure 5. Within-infant weighted Bray-Curtis distances by age. Distances between samples collected ≥6 months were smaller than between samples collected <6 vs ≥6 months (*pairwise Wilcoxon rank-sum p<0.0001), suggesting a difference in beta diversity by age. There was a trend (p=0.07) comparing distances between samples <6 vs ≥6 months.

HIV exposure was associated with lower virome richness but not Shannon diversity, while both richness & diversity increased with age, mixed feeds.

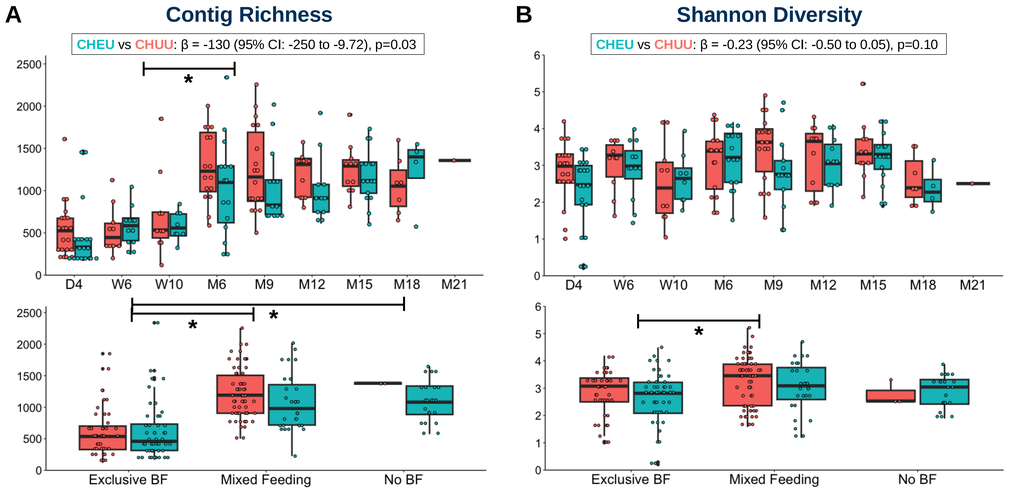


Figure 3. Contig richness (A) and Shannon diversity index (B) by HIV exposure and study visit or feeding mode. Kruskal-Wallis p<0.001 for all comparisons by study visit and feeding. *Pairwise Wilcoxon rank-sum p≤0.001 adjusted for multiple testing.

Relative abundance of several *Anelloviridae* taxa was lower among CHEU than CHUU.

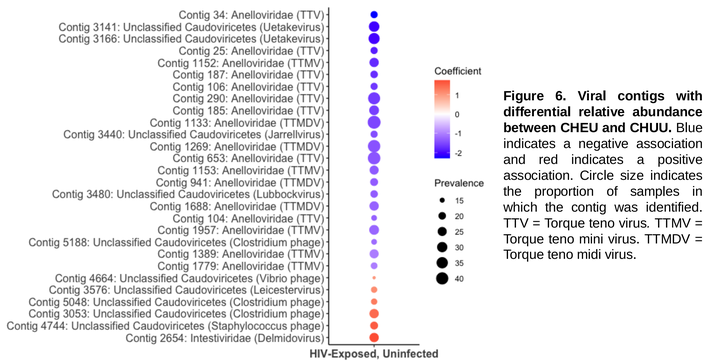


Figure 6. Viral contigs with differential relative abundance between CHEU and CHUU. Blue indicates a negative association and red indicates a positive association. Circle size indicates the proportion of samples in which the contig was identified. TTV = Torque teno virus. TTMV = Torque teno mini virus. TTMDV = Torque teno midi virus.

Conclusions

- Healthy CHEU and CHUU in this cohort had a similar gut virome composition, suggesting that HIV exposure is not a major determinant of the infant virome when mothers receive optimized ART before or in early pregnancy.
- The associations between age, introduction of foods other than breast milk and virome richness/diversity are consistent with previous studies in high income countries, suggesting patterns of virome development are similar globally.
- Further research is needed to understand the why CHEU had lower virome richness and relative abundance of several anellovirus taxa, and to assess whether exclusive breastfeeding contributes to this difference.

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