# *Breast milk Microbiome/Virome of HIV+ Kenyan Women is Not Altered by Immunosuppression*

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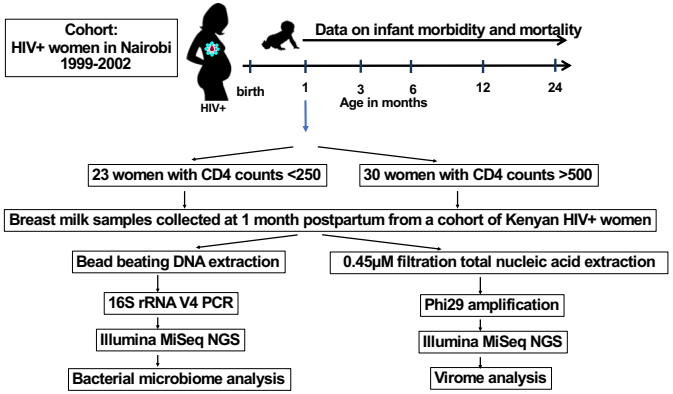

## BACKGROUND

Breast milk (BM) harbors a diverse community of bacteria (microbiome) and viruses (virome) that are transmitted from mother-to-infant during breastfeeding and are important for establishing a healthy infant gut flora. Whether the breast milk microbiome and virome of women living with HIV are altered by immunosuppression and influence morbidity in HIV-exposed infants are unknown.

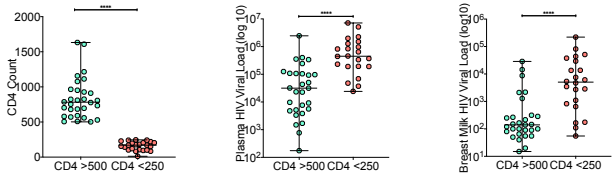
## HYPOTHESIS

We hypothesized that immunosuppression, as measured by low maternal CD4 count, alters the breast milk virome and microbiome.

## COHORT & METHODS

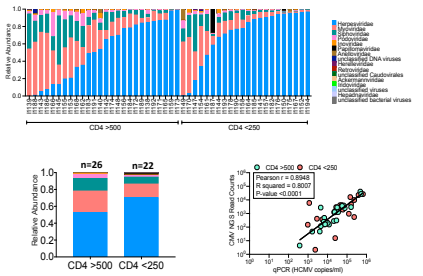



### CD4 counts, plasma and breast milk HIV RNA levels in women with high versus low CD4.

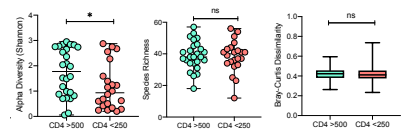



## RESULTS

### Virome

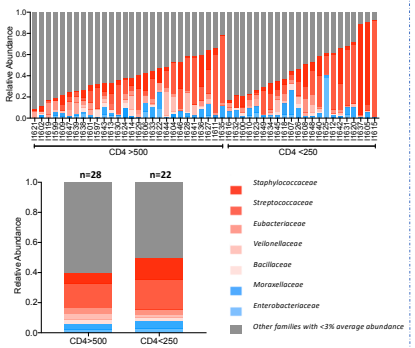



**Relative abundance of viral families in breast milk of women with high vs low CD4 count**. Relative abundance of viral families in breast milk of individual Kenyan women living with HIV (top). Average relative abundance of viral families in women with high vs low CD4 (bottom left). Herpesviridae and bacteriophages dominate the breast milk virome in this cohort of Kenyan women living with HIV. CMV was the predominant herpesvirus as quantified by virome sequencing read count, which was verified by an independent qPCR: r=0.89, p<0.0001 (bottom right). The average proportion of virome sequences that were CMV was lower in women with CD4>500 (53%) compared to women with CD4 <250 (71%, p=0.05).

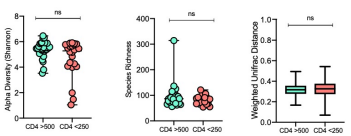



**Comparisons of the alpha diversity (left), species richness (center), and beta-diversity (right) in breast milk virome of women with high vs low CD4 count.** There was a significant difference in breast milk virome alpha diversity (p=0.037), but no significant difference in richness (p>0.68), or beta-diversity (p>0.30) of women with CD4>500 vs CD4<250

### Bacterial Microbiome

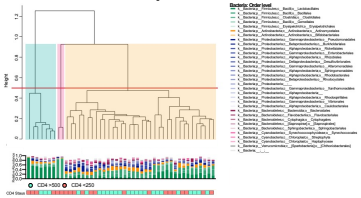



**Relative abundance of bacterial families in breast milk of women with high vs low CD4 count.** Relative abundance of bacterial families in breast milk of individual Kenyan women living with HIV (top). Average relative abundance of bacterial families in women with high vs low CD4 (bottom). The core bacterial microbiome in the breast milk was composed of *Streptococcaceae* (18.1%), *Staphylococcaceae* (10.1%), *Moraxellaceae* (4.1%) and *Eubacteriaceae* (3.6%) families.



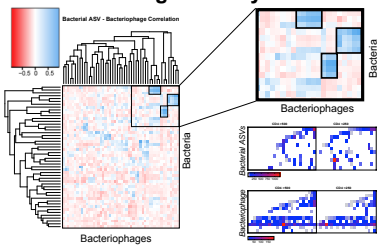

**Comparisons of the alpha diversity (left), species richness (center), and beta-diversity (right) in bacterial microbiome of women with high vs low CD4 count.** There was no significant difference in the breast milk bacterial microbiome richness (p>0.65), alpha-diversity (p>0.14) and beta-diversity (p>0.17) of women with CD4>500 vs CD4<250.

### Bacterial profiles in breast milk cluster independent of CD4 count




**Relative abundance of Order-level bacteria from breast milk sequence data** was clustered using Euclidean distances (top) with a 0.5 cutoff to separate clusters with similar bacterial profiles. Relative abundance of Order-level bacterial data (middle) and CD4 count (bottom) is shown for individual participants. Breast milk profiles appear independent of CD4 count. Euclidean clusters include: Green shaded box (left), *Lactobacillales* dominate. Red shaded box (middle), *Bacillales* dominate. Yellow shaded box (right), *Lactobacillales, Bacillales, Clostridiales, Actinomycetales, Psuedomonadales.*

### Transkingom Analysis




**Transkingdom interactions between co-occurring bacteriophages and bacteria.** Pearson correlation was used to assess relationship between abundance of amplicon sequence variants (ASVs) of bacterial species (y-axis) and bacteriophage species (x-axis) as denoted by shading from red to blue as in key. Clusters outlined in black show strong correlations, including correlations between *Pseudomonadales* ASV with *Pseudomonas* bacteriophages (enlarged in upper right). Heatmaps on lower right show individual participant levels of the bacterial variants (top heatmap) and bacteriophages (bottom heatmap) that contribute to highlighted clusters above. Patterns do not appear to be associated with CD4 count.

## CONCLUSIONS

- **Breast milk harbors a core bacterial microbiome composed of *Streptococcaceae, Staphylococcaceae, Moraxellaceae* and *Eubacteriaceae.***
- **CMV and bacteriophages dominated the breast milk virome in this HIV-infected Kenyan cohort.**
- **In this cohort of Kenyan women with HIV during the pre-ART era, the bacterial microbiome and virome in breast milk from 1 month postpartum were not significantly influenced by immunosuppression.**


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