Viral and Microbial Environment in the Male Genital Tract of People Living with HIV

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BACKGROUND

- HIV persists in cellular reservoirs despite suppressive antiretroviral therapy (ART), a barrier to HIV cure.
- **The male genital tract (MGT) is an immune-privileged site, fostering HIV persistence and local inflammation.**
- Co-infecting viruses (CMV and EBV) and genital microbiome may influence HIV persistence**.**

Study Question: How do co-infecting viruses (CMV, EBV) and the microbiome impact HIV persistence in male genital tract (MGT) tissue reservoirs?

METHODS

- **Study Participants (n=18):** Men with HIV, average age ~65 years, 88% virally suppressed on ART, average CD4+ T-cell count of ~203 cells/μL, enrolled in the “Last Gift” rapid research autopsy program*.
- **Samples:** 69 MGT tissue samples (17 Epididymis, 18 Prostate, 17 Seminal Vesicle, 17 Testes).
- **Timing:** Rapid autopsy* (<6 hrs post-mortem) from 2017-2024.
- **Measurements in all tissues:**
  - HIV/CMV/EBV DNA and HIV RNA via ddPCR.
  - Bacteriome via 16S rRNA sequencing.
- **Statistics:**
  - Mixed-effect and generalized linear models to assess associations between CMV, EBV, and relative abundance of taxa (predictors) with HIV DNA and RNA levels.
  - P-values adjusted for multiple comparisons.

The Last Gift study* at UC San Diego explores HIV persistence in terminally ill individuals, analyzing tissues inaccessible in living patients to gain insights into viral reservoirs.



Participants' contributions extend beyond their lifetimes, leaving a legacy that supports HIV research for decades.

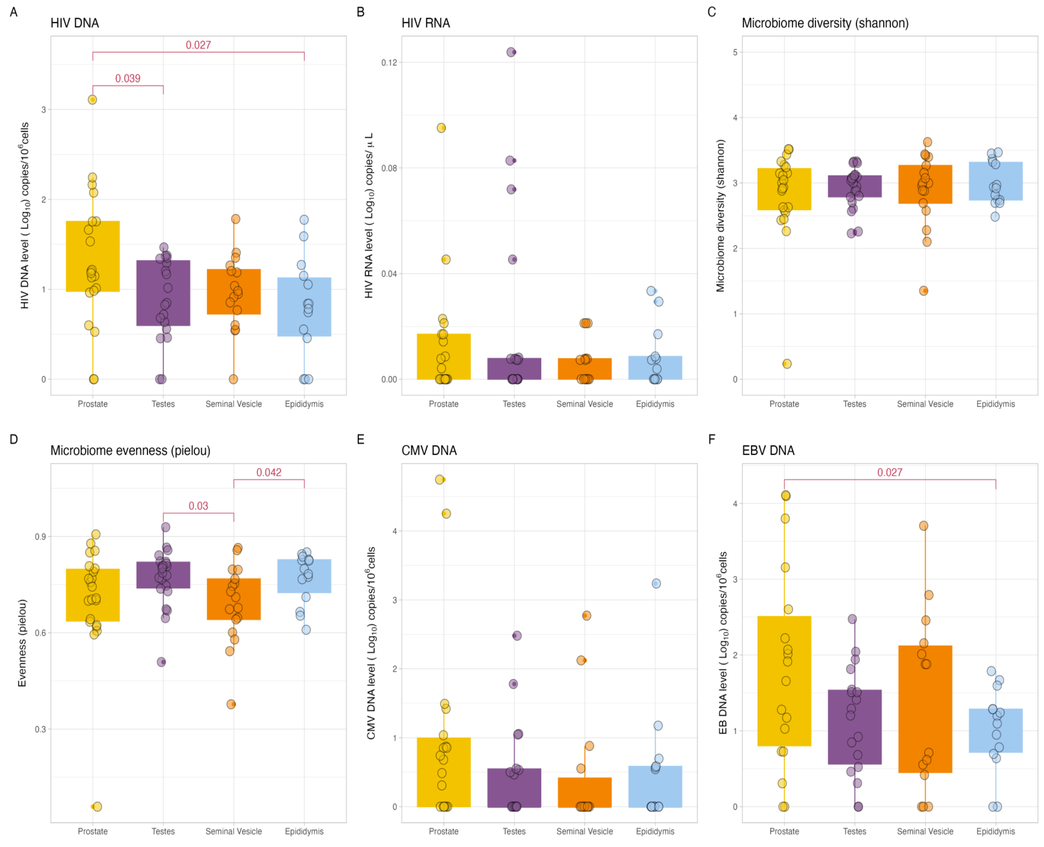
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Acknowledgements: This work was supported by the James B. Pendleton Charitable Trust and grants from the National Institutes of Health including AI036214 (*Translational Virology Core),* AI007384 (HOPE T32 Training Grant),

In the male genital tract, EBV DNA is associated with higher HIV DNA and RNA levels, while *Rhodospirillaceae* and *Pseudonocardia* are associated with higher HIV RNA.

Viruses and microbiome alterations in the MGT may drive local inflammation, fostering HIV persistence and guiding therapeutic strategies.

Figure 1: Comparison of HIV DNA and RNA levels, microbiome diversity (Shannon), microbiome evenness (Pielou), and CMV and EBV DNA levels across MGT tissues (Prostate, Testes, Seminal Vesicle, and Epididymis). P-values for statistically significant differences between tissues in red.



HIV DNA and RNA are detected across all male genital tract tissues, with variations in microbiome composition, viral co-infections (EBV, CMV) and microbiome composition, highlighting the complexity of this persistent reservoir.

RESULTS

- HIV DNA detected in 42 (61%) of samples (~54.3 copies/10^6 cells) and RNA in 27 (39%) samples (~0.067 copies/10^6 cells). Significant variations observed in HIV DNA between Prostate and Testes (p = 0.039) and Prostate and Epididymis (p = 0.027) (Figure 1).
- CMV and EBV detected in 54% and 84% of samples respectively (Table 1).
- **EBV DNA levels associated with higher HIV DNA** (p = 0.002) **and RNA levels** (p = 0.049).
- Adjusting for multiple comparisons, ***Rhodospirillaceae* and *Pseudonocardia* were significantly associated with elevated HIV RNA levels (p = 0.036)** but not DNA.

Tissue	CMV Detected	EBV Detected
Epididymis	9 (53%)	14 (82%)
Prostate	12 (67%)	15 (83%)
Seminal Vesicle	12 (70%)	14 (82%)
Testes	9 (53%)	15 (88%)

Table 1: Prevalence of CMV and EBV across MGT tissue samples collected detected via ddPCR.

Predictor	Outcome	Effect Estimate	Adjusted p-values
EBV DNA	HIV DNA	0.353	**0.002**
	HIV RNA	0.009	**0.049**
CMV DNA	HIV DNA	0.021	0.859
	HIV RNA	0.002	0.694
Rhodospirillaceae	HIV RNA	139.614	**0.036**
Pseudonocardia	HIV RNA	29.504	**0.036**

Table 2: Associations between EBV DNA, CMV DNA, and microbial genera (predictors) with cellular HIV DNA and RNA levels (outcomes) across all MGT tissues derived from mixed effect and generalized linear models adjusted for age, duration of HIV infection, CD4+ nadir, latest viral load and CD4+ T cell counts at the time of death. P-values for microbiome were adjusted for multiple comparisons. Only significant genera shown for microbiome. Significant p-values are bold.

CONCLUSIONS

- The MGT remains a key HIV reservoir despite suppressive ART.
- These findings highlight the need to explore viral and microbial influences on HIV persistence for future cure strategies.
- Ongoing analyses of additional MGT and bladder specimens aim to further elucidate the mechanisms of HIV persistence and expand our understanding of its reservoir dynamics.