

# Recovery of Infectious Human Immunodeficiency Virus Type-1 (HIV-1) from the Oropharynx: Implications for Oral Transmission of HIV-1

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## ABSTRACT

**Background:** Oral-genital transmission of HIV-1 occurs and HIV-1 RNA is readily detected in the saliva; however, culture of HIV-1 from the saliva has been generally unsuccessful (<1% of specimens) due to inactivation of virus by saliva. Our objective was to describe the predictors and variability of HIV-1 RNA viral load (VL) in the pharynx and the recovery of cultivable HIV-1 from oropharyngeal surfaces.

**Methods:** Sexually active HIV positive men from Seattle and Lima, Peru who have sex with men, without bacterial STDs, were evaluated prospectively at 0, 2 and 4 weeks to assess viral load in blood and swab specimens obtained from the posterior oropharynx. HIV-1 RNA was quantified using a commercial PCR amplification assay (Roche HIV-1 Monitor). A subset of 17 men was evaluated for recovery of infectious virus from blood, and tonsil and buccal surfaces.

**Results:** The median CD4 count of 64 participants was 290 cells/ml; 45% were currently receiving antiretrovirals. The median baseline VL was similar in blood plasma (4.24 log<sub>10</sub> HIV-1 RNA copies/ml) and the pharynx (4.22 log<sub>10</sub>). The within subject variability of pharyngeal VL (weighted average SD .404 log<sub>10</sub>) was greater than that of blood (weighted average SD .840 log<sub>10</sub>). By generalized estimating equations, each 1.0 log<sub>10</sub> increase in plasma VL was associated with a .323 log<sub>10</sub> increase in pharyngeal VL (P<0.001), and antiretroviral therapy (.636 log<sub>10</sub>) and tonsillectomy (.329 log<sub>10</sub>) were associated with reductions in pharyngeal VL (P=0.008 and 0.09, respectively). HIV-1 was cultured from the posterior oropharyngeal surface from 4 (24%) of 17 men; HIV was not cultured from the buccal mucosal surface. Median mucosal RNA VL was 5.85 log<sub>10</sub> in culture positive men versus 4.69 log<sub>10</sub> in culture negative men (P=0.089).

**Conclusions:** Pharyngeal VL is lower among men with prior tonsillectomy and increased among men with higher plasma viral load. Our results demonstrate that infectious HIV-1 can be recovered from the oropharyngeal mucosal surface. These data indicate a potential for oral transmission of HIV-1. Sexual acts that facilitate contact with the posterior oropharyngeal mucosal surface may be associated with increased risk of oral transmission as has been reported for HHV-8, although additional behavior data are needed.

• Lower limits of detection (LOD) were 400 and 1,200 copies/ml for blood plasma and pharyngeal and buccal mucosal samples, respectively. HIV RNA viral load was adjusted for specimen dilution.

### Statistical methods.

• Censoring: undetectable measurements were assumed to be halfway between zero and lower LOD (plasma= 2.30 log<sub>10</sub> c/ml; oropharynx=2.78 log<sub>10</sub> c/ml).

• Within-subject variability was calculated over 2-3 visits.

• Chi-square tests (with Fisher's exact where appropriate) were used for analysis of dichotomous variables. The nonparametric tests, Kruskal-Wallis and Wilcoxon Signed Ranks tests, were used for analysis of continuous variables.

• Generalized estimating equations (GEE) were used to evaluate factors associated with HIV viral load, while controlling for covariates during repeated visits by the same subjects.

## BACKGROUND

• HIV-1 RNA is often detected, but HIV is rarely cultured from the oropharynx perhaps due to inactivation by saliva.<sup>1,2</sup>

• Anatomic sites of HIV replication in the oropharynx have not been determined, although *in situ* hybridization of tonsillar biopsy specimens have shown HIV RNA and DNA.<sup>3</sup>

• Determination of factors associated with increased RNA viral load and recovery of infectious HIV from the oropharynx may have implications for understanding oral transmission of HIV.

## STUDY OBJECTIVES

• Characterize factors associated with increased pharyngeal HIV viral load.

• Determine feasibility of HIV-1 recovery in culture from several sites in the oropharynx and factors associated with viral recovery.

## METHODS

• HIV+ men who have sex with men (MSM) were studied prospectively over 3 visits (0, 2 wks, and 4 wks) in Seattle, WA & Lima, Peru. A subset of 17 men with high pharyngeal viral loads were evaluated at a fourth visit approximately one year later.

• Screening for bacterial STDs performed at visit 1; men with symptoms or confirmed STDs were treated. This analysis includes only asymptomatic men without STDs.

• At each visit, HIV RNA was quantified by RT PCR (Amplicor™, Roche) in blood and from secretions collected by swab from the posterior pharynx. At the one year follow-up visit, pharyngeal swab specimens were obtained for RNA level and infectivity by a mixed lymphocyte coculture method.<sup>4</sup> In Seattle men, blood and an additional swab of the buccal mucosa were collected for determination of HIV RNA level and culture.

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## RESULTS

**Table 1. Clinical and behavioral data for 64 MSM without clinical or laboratory evidence for bacterial sexually transmitted infection (STI)**

	All Men (64)	Seattle (33)	Peru (31)
Median Age, years (range)	35.5 (23-57)	39 (25-57)	27 (23-50)†
Median CD4 count, cells x 10 <sup>6</sup> (range)	290 (10-1105)	313 (14-933)	269 (10-1105)
Median Baseline HIV RNA log copies/ml:			
Plasma (range) ‡	4.24 (2.30-6.22)	3.12 (2.30-5.86)	4.75 (2.30-6.22)†
Number with detectable plasma RNA (%)	46/63 (73)	17/32 (53)	29/31 (94)†
Pharynx (range) ‡	4.22 (2.78-6.05)	3.11 (2.78-5.77)	4.86 (2.78-6.05)†
Number with detectable pharyngeal RNA (%)	43/62 (67)	16/31 (49)	27/31 (87)†
Patients with tonsillectomy (%)	15/57 (26.3)	11/26 (42.3)	4/31 (12.9)†
Antiretroviral Therapy:			
Any ART (%)	29 (45.3)	24 (72.7)	5 (16.1)†
Dual NRTI (%)	21 (32.8)	16 (48.5)	5 (16.1)†
NNRTI/dual NRTI (%)	5 (7.8)	5 (15.2)	0
PI-containing regimen (%)	20 (31.3)	16 (48.5)	4 (12.9)†
Median no. SP, past yr. (range)	4 (0-130)	6 (1-130)	3 (0-100)

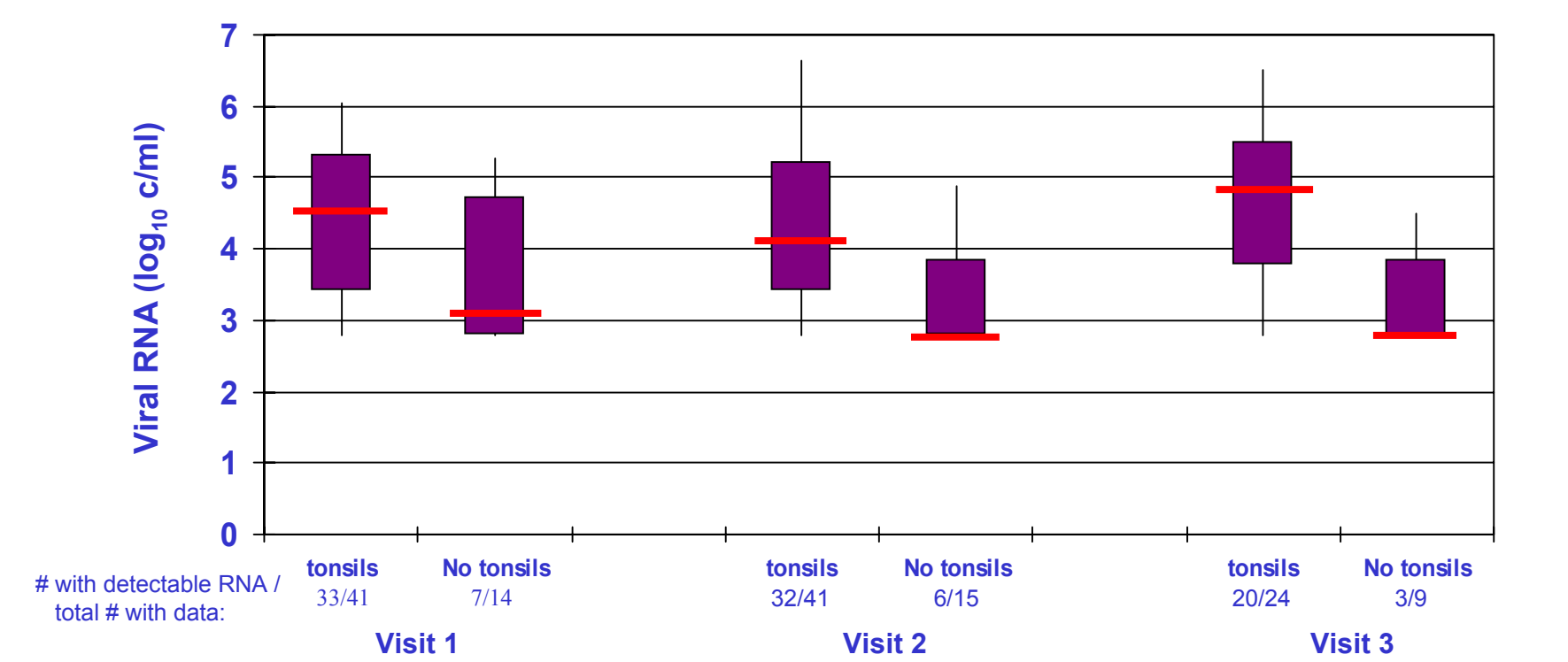
† p-value < 0.05 when compared to Seattle men (Kruskal-Wallis test of medians, Chi-square with Fisher's exact test)

‡ Weighted average of the within-subject variability was SD= .404 log<sub>10</sub> for plasma and SD=.840 log<sub>10</sub> for pharynx.

NOTE: ART, antiretroviral therapy; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; SP, sex partners.

**Comment:** There was a statistically significant study site effect between Seattle and Lima with respect to age, treatment, viral load and tonsillectomy. Within-subject RNA levels were 2.1-fold more variable in the pharynx than blood plasma.

**Figure 1. Median pharyngeal HIV-1 viral loads by visit with respect to tonsillectomy status**



Note: Because at least a quarter of samples in patients with tonsillectomy had undetectable VL, the box plots have no "bottom whiskers" (i.e., the 25<sup>th</sup> percentile and lower limit of the range are the same number—namely, half of the lower limit of detection for the assay).

**Comment:** The detected range of HIV-1 RNA were different between men with and without tonsils uncontrolled for ART

**Table 2. Multivariate model of predictors of pharyngeal HIV-1 RNA Shedding**

Factor	β	p-value	95% CI β
Log <sub>10</sub> plasma RNA VL†	0.323	<0.001	0.156-0.490
Antiretroviral therapy	-0.636	0.008	-1.056 -0.215
History of tonsillectomy	-0.329	0.09	-0.717- 0.060
Constant	3.189		

†For each 1.0 log<sub>10</sub> plasma RNA VL there was a .323 log<sub>10</sub> (2.1 fold) increase in pharyngeal viral load

**Comment:** Age, CD4 and Site did not significantly change the multivariate model, although they were significantly associated with pharyngeal VL in univariate models (p<0.001, 0.004, <0.001, respectively)

**Table 3. Oro-pharyngeal HIV RNA Levels and Pharyngeal and Blood HIV Culture Results for a subset of 17 Subjects**

Patient ID	Site	Tonsils	Pharyngeal RNA level	Buccal Swab RNA level	Pharyngeal HIV Culture Result (phenotype)	Blood HIV Culture Result (phenotype)
1003	Seattle	Yes	3.89	3.13	Negative	Negative
1014	Seattle	No	3.44	3.38	Negative	Negative
1016	Seattle	Yes	3.08	2.91	Negative	Positive (NSI)
1021	Seattle	Yes	5.17	3.12	Negative	N/A
1024	Seattle	Yes	5.37	3.78	Negative	Positive (SI)
1026	Seattle	Yes	3.08	3.31	Negative	Positive (SI)
1039	Seattle	Yes	6.29	3.66	Negative	N/A
1049	Seattle	Yes	3.42	2.98	Negative	Positive (NSI)
2002	Peru	Yes	5.41	N/A	Negative	N/A
2004	Peru	Yes	2.96	N/A	Negative	N/A
2012	Peru	Yes	4.69	N/A	Negative	N/A
2013	Peru	Yes	5.18	N/A	Positive (indet.)	N/A
2017	Peru	Yes	5.63	N/A	Negative	N/A
2019	Peru	Yes	5.78	N/A	Positive (NSI)	N/A
2024	Peru	Yes	5.97	N/A	Positive (NSI)	N/A
2025	Peru	Yes	5.93	N/A	Positive (NSI)	N/A
2051	Peru	Yes	6.04	N/A	Negative	N/A

Note: No buccal swabs were culture positive

RNA levels in log copies/ml

N/A: not available (buccal swabs only obtained for Seattle men); indet., indeterminate

**Comment:** HIV was only isolated from pharyngeal swabs, not from buccal swabs or saliva (data not shown).

**Table 4. Comparative results (baseline versus 1 year later) for 17 MSM who had pharyngeal HIV cultures**

Patient ID	CD4 Count	ART at follow-up	Time interval from baseline (weeks)	Plasma viral load		Pharyngeal RNA level		Log difference in pharyngeal RNA level‡
				Baseline	Year 1†	Baseline	Year 1	
1003	569	2NRTI/NNRTI	79	3.20	2.94	4.05	3.89	-0.16
1014	587	No	64	4.19	4.34	4.60	3.44	-1.16
1016	600	2NRTI/PI	64	2.30	2.30	4.95	3.08	-1.87
1021	192	No	62	4.87	4.76	4.56	5.17	0.61
1024	93	No	57	4.89	6.03	3.55	5.37	1.82
1026	395	2NRTI/PI	59	5.54	2.30	4.47	2.78	-1.69
1039	272	No	47	4.91	5.23	5.77	6.29	0.52
1049	490	No	34	3.41	3.59	3.30	3.42	0.12
2002	361	No	49	4.34	N/A	5.67	5.41	-0.25
2004	178	2NRTI/PI	49	5.47	N/A	5.45	2.78	-2.68
2012	223	No	48	4.50	N/A	5.51	4.69	-0.82
2013	202	No	48	4.75	N/A	5.16	5.18	0.02
2017	315	No	48	4.84	N/A	6.48	5.63	-0.85
2019	427	No	48	4.66	N/A	5.98	5.78	-0.20
2024	269	2NRTI	47	4.24	N/A	5.95	5.97	0.02
2025	250	No	47	4.76	N/A	5.08	5.93	0.84
2051	32	No	36	5.24	N/A	6.31	6.04	-0.27

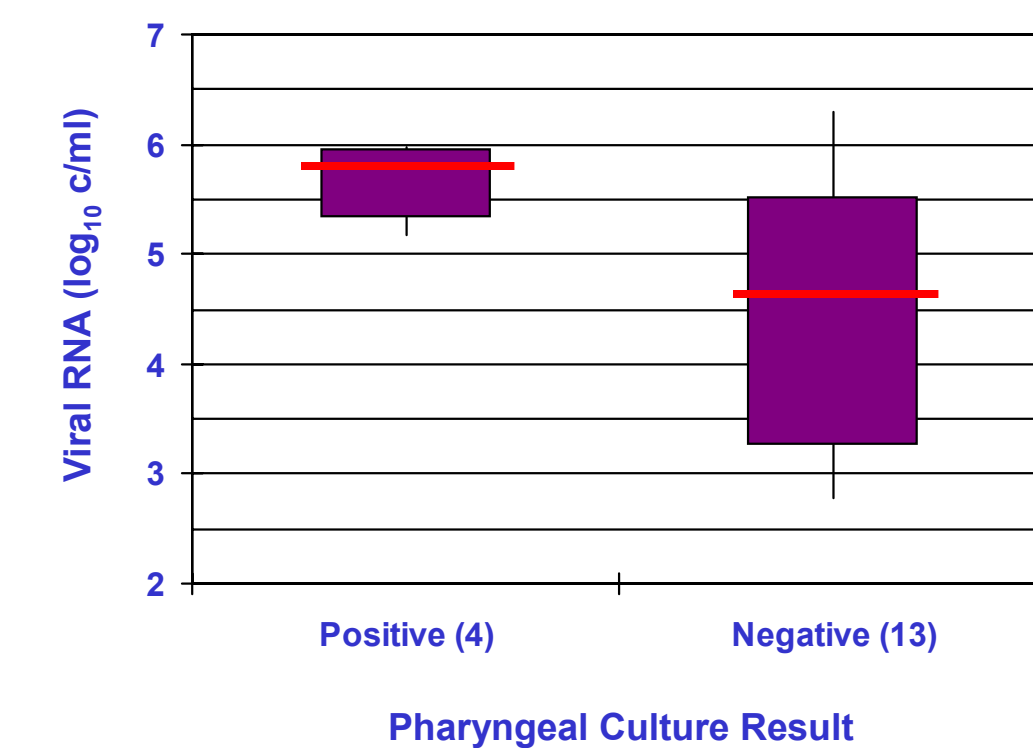
† Wilcoxon test comparing baseline to year 1 Plasma VL shows no significant difference in distribution of median values (p=0.735).

‡ P=0.173, for Wilcoxon test.

Plasma viral loads and pharyngeal RNA levels are log copies/ml

2NRTI, two nucleoside reverse transcriptase inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; N/A, not available

**Figure 2. Median pharyngeal HIV viral loads by culture result**



**Comment:** HIV was more likely to be isolated from the pharyngeal swabs of men with high pharyngeal RNA (p=0.089)

## MAIN FINDINGS

• Tonsillectomy and ART are associated with a reduction in pharyngeal viral load.

• Within-subject RNA levels were at least 2-fold more variable in the pharynx than blood plasma.

• HIV was cultured from the posterior pharynx in 4 of 17 men (24% of those sampled) and none of samples from the buccal mucosa or saliva.

• Patients with positive pharyngeal cultures had high levels of pharyngeal RNA.

## CONCLUSIONS

• Pharyngeal HIV shedding is higher in persons with tonsils and with higher plasma RNA levels; however even persons without tonsils have detectable pharyngeal HIV RNA.

• Anatomically, the posterior oropharynx appears to harbor higher levels of HIV and more viable virus than the buccal mucosa. This difference is likely due to the proximity of lymphoid tissue in the posterior oropharynx.

• The detection of culturable HIV in the posterior pharynx may indicate the potential for oral transmission of HIV.

• Sexual acts that facilitate contact with the posterior oropharyngeal mucosal surface may be associated with increased risk of oral transmission as has been reported for HHV-8,<sup>5</sup> although additional behavior data are needed.

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