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HIV-1 subtype C is not associated with higher risk of heterosexual HIV-1 transmission: a multinational study among African HIV-1 serodiscordant couples

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Abstract

Background—HIV-1 subtype C has emerged as the most prevalent strain of HIV-1 worldwide, leading to speculation that subtype C may be more transmissible than other subtypes. We compared the risk of HIV-1 transmission for subtype C versus non-C subtypes (A, D, G and recombinant forms) among heterosexual African HIV-1 serodiscordant couples.

Methods—We conducted a nested case-control analysis using data from two prospective cohort studies of heterosexual HIV-1 serodiscordant couples from 6 countries in eastern and southern Africa. Cases (N=121) included incident HIV-1 transmissions that were established as linked

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within the serodiscordant partnership by viral sequencing; controls (N=501) were non-transmitting HIV-1 infected partners. Subtype was determined for partial *env* and *gag* genes. Multiple logistic regression controlled for age and gender of the HIV-1 infected partner and self-reported unprotected sex. Plasma and genital HIV-1 RNA concentrations were compared between subtype C and non-C subtypes using generalized estimating equations.

Results—HIV-1 subtype C was not associated with increased risk of HIV-1 transmission compared to non-C subtypes: *env* adjusted odds ratio (adjOR) 1.14 (95% confidence interval [CI] 0.74–1.75, $p=0.6$) and *gag* adjOR 0.98 (95% CI 0.63–1.52, $p=0.9$). Plasma and genital HIV-1 RNA levels did not differ significantly for subtype C versus non-C.

Conclusion—In a geographically diverse population of heterosexual African HIV-1 serodiscordant couples, subtype C was not associated with greater risk of HIV-1 transmission compared to non-C subtypes, arguing against the hypothesis that subtype C is more transmissible compared to other common subtypes.

Keywords

HIV-1 subtype; transmission; serodiscordant couples; Africa

Introduction

HIV-1 subtype C accounts for nearly half of all HIV-1 infections worldwide, primarily due to its predominance in sub-Saharan Africa, where the majority of HIV-1 infections occur^{1, 2}. The explosion of heterosexually transmitted HIV-1 throughout southern Africa in the 1990s was almost exclusively due to HIV-1 subtype C, leading some to hypothesize that subtype C might be more transmissible compared to other subtypes^{3–6}. Laboratory studies have suggested molecular and genetic characteristics of subtype C that could promote more efficient transmission^{7–9}. However, clear evidence for differential transmissibility of HIV-1 subtypes in population-level epidemiological studies has not been shown^{10–12}. HIV-1 genetic diversity, including subtype diversity, poses a challenge to the development of a globally-effective HIV-1 vaccine¹³, and subtype-related differences in HIV-1 transmission, if present, would be a critical consideration in the selection of vaccine antigens^{2, 14}.

Epidemiologic studies directly measuring the relationship between HIV-1 subtype and heterosexual transmission risk have been challenging for two main reasons. First, prospective studies of HIV-1 transmission require following large numbers of HIV-1 infected persons and their uninfected sexual partners in order to identify rates of HIV-1 transmission occurring within the partnerships. Second, HIV-1 subtypes tend to be geographically specific, and thus studies must include populations from multiple regions in order to have sufficient subtype variation for comparison of transmission risk. Several studies of mother-to-child transmission have had mixed results when comparing vertical HIV-1 transmission by subtype^{15–18}. Even fewer studies of subtype and transmission exist for heterosexual transmission. One HIV-1 serodiscordant couples study in Uganda found higher transmission risk for subtype A compared to D¹⁹, but subtype C was not present in the study population. Another study of serodiscordant couples in Zambia found subtype C in 95% of genetically-linked transmissions²⁰, but the Zambian epidemic is predominantly

subtype C and thus comparing transmission rates to other subtypes was not possible in that study. In the present study, among a multinational population of heterosexual HIV-1 serodiscordant couples from eastern and southern Africa, our aim was to assess whether subtype C, compared with non-C subtypes, was associated with greater HIV-1 transmission risk.

Methods

Study Population

We conducted a nested case-control study using data from two prospective cohort studies of African HIV-1 serodiscordant couples. Between November 2004 and April 2007, 3408 heterosexual HIV-1 serodiscordant couples from 6 African countries (Botswana, Kenya, South Africa, Tanzania, Uganda, and Zambia) were enrolled into the Partners in Prevention HSV/HIV Transmission Study, a randomized, double-blind, placebo-controlled clinical trial of herpes simplex virus type 2 (HSV-2) suppressive therapy to reduce HIV-1 transmission, as previously described²¹. Eligible couples were at least 18 years of age, reported at least three vaginal sex acts in the three months prior to enrollment, and intended to remain as a couple. At enrollment, all HIV-1 infected partners were HSV-2 seropositive, had CD4 counts ≥ 250 cells/ μ L (making them ineligible for antiretroviral therapy (ART) under the national guidelines of the study countries at that time), and were not currently taking ART. HSV-2 suppressive therapy was found not to reduce HIV-1 transmission within the partnerships²². In a parallel study at two sites (Kampala, Uganda and Soweto, South Africa), an additional 485 HIV-1 serodiscordant couples were enrolled into an observational study of immune correlates of HIV-1 protection (Couples Observational Study)²³. Similar to the clinical trial cohort, participants were ≥ 18 years of age and sexually active and HIV-1 seropositive partners were not using ART. In both cohorts, initially HIV-1 uninfected participants were followed quarterly, with HIV-1 serologic testing.

Protection of Human Subjects

All participants received HIV-1 and risk-reduction counseling (both individually and as a couple), free condoms, and treatment for sexually transmitted infections (STIs), according to WHO guidelines. Written, informed consent was obtained from all participants. The study protocols were approved by the University of Washington Human Subjects Review Committee and ethical review committees at each of the study sites.

Selection of cases and controls

Cases were defined from our primary cohort studies as all HIV-1 infected partners of HIV-1 seroconverters, limited to those couples in which it was determined, through viral genetic linkage, that HIV-1 transmission occurred within the partnership (as opposed to from an outside partner)²⁴. A total of 121 cases were included: 106 from the Partners in Prevention HSV/HIV Transmission Study and 15 from the Couples Observational Study. Controls were selected randomly, in proportion to research site and gender distribution of each primary study, from non-transmitting HIV-1 infected partners to achieve a 1:4 case to control ratio. Since HIV-1 subtype was expected to be correlated with site, given the geographic

association of HIV-1 subtypes in Africa, the proportional sampling of controls was used to select controls representative of the full cohort. In total, 501 controls were selected.

Laboratory Testing

HIV-1 seroconversion of initially HIV-1 uninfected partners was determined by quarterly serologic testing using dual rapid HIV-1 antibody tests with confirmatory HIV-1 enzyme immunoassay (EIA), Western blot, and plasma HIV-1 RNA detection. Plasma HIV-1 RNA levels for HIV-1 infected partners were quantified using the COBAS Ampliprep/COBAS TaqMan real-time HIV-1 RNA assay version 1.0 (Roche Diagnostics, Indianapolis, IN). Plasma HIV-1 RNA viral loads were assessed at enrollment and visit months 3, 6, 9, 12 and study exit for the Partners in Prevention HSV/HIV Transmission Study and at enrollment only for the Couples Observational Study. Genital HIV-1 RNA was quantified using the TaqMan assay from samples collected at a single study visit in the Partners in Prevention HSV/HIV Transmission Study: seminal plasma for HIV-1 infected men, collected at any visit 3 months after enrollment and endocervical swabs for HIV-1 infected women, collected at a visit 6 months after enrollment²⁵. All viral loads were log₁₀ transformed, and results below the limit of quantification (<240 copies/mL) were assigned a value of half the limit.

Viral sequencing using blood plasma was performed on partial HIV-1 *env* (C2-V3-C3) and *gag* (p17–p24) genes using samples collected at the first post-seroconversion study visit for cases and at the last follow-up visit for controls. Genetic linkage of HIV-1 transmission events was based on phylogenetic analysis and posterior probability of linkage using pairwise nucleotide distances between sequences²⁴.

Subtypes were determined by the REGA subtype tool version 2.0 (<http://dbpartners.stanford.edu/RegaSubtyping/>). Sequence data were provided to GenBank and accession numbers are pending.

Data analysis

We compared HIV-1 transmission risk in cases versus controls between subtype C and all non-C subtypes (including A, D, G, and recombinants) separately for both *env* and *gag*. All cases had subtype information available in *gag*, *env* or both gene regions, but among controls, 43/501 (8.6%) were missing all subtype data, including 34/332 (10.2%) from eastern African and 9/169 (5.3%) from southern Africa, due to low HIV-1 plasma viral loads preventing adequate viral amplification. To avoid bias because of control exclusion due to missing subtype data, we performed multiple imputation with 20 datasets imputed using Markov chain Monte Carlo methods²⁶.

To assess differences in HIV-1 transmission between subtype C to non-C subtypes, we performed a standard case-control analysis using logistic regression, analyzing the 20 imputed datasets and combining the results to produce standard estimates and 95% confidence intervals. All models were adjusted for gender and age of the HIV-1 infected partner and self-reported unprotected sex in the month prior to study enrollment. We assessed other variables for potential confounding, some of which may reflect regional

differences, including circumcision status of male HIV-1 uninfected partners, duration of partnership, number of children, presence of sexually transmitted infections, any ART initiation during follow-up by HIV-1 infected partners, and CD4 count of HIV-1 infected partners; however, none of these factors substantially changed the effect estimates and thus were not included in the final models. In additional analyses, we further adjusted for baseline plasma HIV-1 RNA concentrations to assess the association of subtype C and HIV-1 transmission independent of plasma viral load. With the available sample size, we estimated we would have 80% power to detect a 1.85-fold increased odds of HIV-1 transmission for subtype C versus non-C at the alpha 0.05 level.

In addition to the nested case-control analysis, in order to incorporate changes in longitudinal covariates, including time-dependent covariates such as plasma HIV-1 RNA and unprotected sex, we also employed a case-cohort analysis, as a secondary analysis. We used Cox proportional hazards analyses, adjusted for gender, age of the HIV-1 infected partner, and longitudinal report of unprotected sex and plasma HIV-1 RNA, to compare transmission by HIV-1 subtype. Case-cohort analysis methods were used ²⁷.

Finally, we compared differences in plasma and genital HIV-1 RNA concentrations between subtype C and non-C subtypes for participants from the Partners in Prevention HSV/HIV Transmission Study. We assessed subtype differences related to longitudinal plasma HIV-1 RNA during study follow-up using repeated measures generalized estimating equations (GEE) models with unstructured correlation matrix, adjusting for gender, age of the HIV-1 infected partner, and unprotected sex. Participants were censored at ART initiation. Genital HIV-1 RNA levels were available at a single time point for 416/624 (66.7%) of the HIV-1 infected partners, and we assessed differences among subtypes using a multiple linear regression for endocervical and semen HIV-1 RNA levels, controlling for age of the HIV-1 uninfected partner, unprotected sex reported at enrollment, and plasma HIV-1 viral load.

All analyses were performed using SAS v.9.2 (SAS Institute, Inc., Cary, N.C.).

Results

Of the 622 HIV-1 infected study participants in the nested case-control cohort, subtype information was available for 579 (93.1%), including all 121 (100.0%) cases and 458/501 (91.4%) controls. The majority of participants were from eastern Africa: 80 (66.1%) cases and 332 (66.3%) controls (Table 1). Most couples (92.0%) were married. Age was similar between cases and controls: median age of cases was 30 years (IQR 26–35) and the median age of controls was 32 years (IQR 26–38). Cases were more likely to report unprotected sex in the month prior to enrollment (52.8% versus 36.2%, $p=0.001$) and less likely to be female (49.6% versus 65.5%, $p<0.001$). The median baseline HIV-1 plasma RNA was significantly higher in cases (4.8 \log_{10} copies/mL, IQR 4.3–5.1) compared to controls (4.2 \log_{10} copies/mL, IQR 3.6–4.8, $p<0.001$).

The most common subtypes were A (*env* 44.0%, *gag* 38.3%) and C (*env* 39.2%, *gag* 39.7%), followed by D (*env* 13.9%, *gag* 11.1%), and G or recombinant subtypes (*env* 2.9%, *gag* 10.9%). Subtype was missing in *env* for 25 (4.3%) and in *gag* for 57 (9.8%). For

participants with both *env* and *gag* subtypes available, concordance between genes was 82.5%, with concordance of 95.5% for subtype C *env* and *gag*. Nearly all participants from southern Africa were infected with subtype C (*env* 98.5%, *gag* 99.5%). In eastern Africa, the predominant subtypes were subtype A (*env* 67.7%, *gag* 59.6%) and subtype D (*env* 21.5%, *gag* 17.4%). The distribution of subtype among cases and controls is shown in Figure 1.

Subtype C and HIV-1 Transmission Risk

In the nested case-control multivariate logistic regression analysis, subtype C was not associated with an increased risk of HIV-1 transmission compared to non-C subtypes, both when considering subtype based on *env* sequencing (adjusted odds ratio [adjOR] 1.14, 95% confidence interval [CI] 0.74–1.75, $p=0.6$) and *gag* sequencing (adjOR 0.98, 95% CI 0.63–1.52, $p=0.9$) (Table 2). Additionally, separate comparisons of subtype C to individual subtypes showed no statistically significant differences in the odds of HIV-1 transmission risk with subtype A (*env* adjOR 1.17, $p=0.5$ and *gag* adjOR 1.09, $p=0.7$) or subtype D (*env* adjOR 1.39, $p=0.3$ and *gag* adjOR 1.79, $p=0.08$). Separate comparisons between subtype C and subtype G or recombinant forms was not possible due to the small number of participants with these subtypes. Further adjusting these same regression models for plasma HIV-1 RNA did not substantially change these results. Additionally, when we compared HIV-1 transmission for subtype A compared to subtype D, we did not find significant differences in *env* (adjOR 1.25, 95% CI 0.66–2.36, $p=0.5$) or *gag* (adjOR 0.89, 95% CI 0.48–1.67, $p=0.7$).

In the case-cohort analysis, which permitted adjustment for unprotected sex as a time-varying covariate, results were similar to those in the nested case-control approach: subtype C was not significantly associated with increased HIV-1 transmission compared to non-C subtypes, in *env* (adjHR 1.56, 95% CI 0.89–2.76, $p=0.1$) or *gag* (adjHR 0.92, 95% CI 0.51–1.67, $p=0.8$). In separate comparisons of HIV-1 transmission risk between subtype C and subtypes A and D, there were also no statistically significant differences for *env* or *gag*. These results were similar with the addition of time-dependent plasma HIV-1 RNA to the models.

Subtype C and HIV-1 Concentrations in Plasma and Genital Secretions

The median plasma HIV-1 RNA during the study was 4.3 log₁₀ copies/mL (IQR 3.7–4.8) among those with *env* subtype C and 4.2 log₁₀ copies/mL (IQR 3.4–4.9) among those with a non-C *env* subtype (Figure 2a; $p=0.2$). The median endocervical HIV-1 RNA for *env* subtype C was 3.3 log₁₀ copies/mL (IQR 2.5–4.0) and for non-C *env* subtypes was 3.4 log₁₀ copies/mL (IQR 2.5–4.0, $p=0.9$) (Figure 2b). The median semen HIV-1 RNA was 2.8 log₁₀ copies/mL (IQR 2.1–3.5) for *env* subtype C and 2.6 log₁₀ copies/mL (IQR 2.1–3.7) for non-C *env* subtypes. Individuals with *env* subtype C did not differ significantly from non-C subtypes by genital viral load in either endocervical fluid ($p=0.9$) or semen plasma ($p=0.6$). Results for *gag* subtype were similar to *env* (data not shown).

Discussion

In this analysis comparing transmitting and non-transmitting HIV-1 serodiscordant couples from eastern and southern Africa, we did not find evidence that subtype C was associated with increased HIV-1 transmission risk, compared with non-C subtypes. Our study population included a wide geographic region with sufficient subtype variation (primarily A, C and D) in order to perform the analyses, and genetic linkage information improved the precision of the results. Previous studies of subtype and HIV-1 transmission have either lacked the subtype diversity to compare subtype C to non-C subtypes or been based on ecological data of prevalent trends in subtype. To our knowledge, this is the first study to provide direct evidence for the question of whether subtype C is associated with increased heterosexual transmission risk compared to other non-C subtypes common in sub-Saharan Africa. Our results do not support the hypothesis that HIV-1 subtype C has greater transmissibility compared with other subtypes.

We conducted both a nested case-control analysis and a longitudinal analysis using a case-cohort study design to assess whether subtype C was associated with an increased risk for HIV-1 transmission. We adjusted for age, gender and reported unprotected sex, and we determined that other factors (e.g., male circumcision status) were not confounding. We did not initially include plasma HIV-1 RNA in our initial models because we hypothesized that if HIV-1 transmission differed by subtype, it could be mediated by subtype-related differences in viral load. However, after finding no association between subtype C and HIV-1 transmission, we further adjusted our models to control for plasma HIV-1 RNA and continued to see no significant relationship between subtype C and HIV-1 transmission risk, compared to non-C subtypes. In both the nested case-control and case-cohort analyses, we also compared subtype C and subtypes A and D separately and found no statistically significant difference in HIV-1 transmission risk.

A limited number of studies have found individuals with subtype C to have higher HIV-1 DNA or RNA concentrations in plasma and genital secretions, which could indicate higher transmission risk^{15, 28, 29}; however, not all studies have found increased HIV-1 concentrations associated with subtype C infection³⁰. In the present study, we assessed whether subtype C was associated with higher plasma and genital HIV-1 RNA concentrations, as a proxy for infectiousness and potential onward transmission. We found no statistically significant differences in plasma and genital HIV-1 RNA levels in participants with subtype C compared to non-C subtypes, further supporting the results of our nested case-control and case-cohort transmission analyses.

The rapid expansion of HIV-1 subtype C throughout sub-Saharan Africa has led some to hypothesize a causal relationship between subtype C and increased HIV-1 transmissibility. However, a combination of other factors may be as likely to contribute to the swift growth of HIV-1 subtype C. A founder effect, which has been hypothesized to explain the dominance of specific subtypes throughout Africa, could be relevant^{31, 32}. Additionally, Tatem et al. recently provided evidence to suggest that regions with greater accessibility allowing for increased mobility, such as in southern Africa, are associated with clusters of similar subtypes throughout the transportation infrastructure³³. Another potential

explanation is that subtype C has shown lower viral fitness, and therefore may result in slower disease progression compared to other subtypes^{11, 34–36}; individuals with a slower progressing disease not only add person-years to prevalence estimates, but also have more opportunity to transmit their infection over a longer period of time. Finally, subtype C may be more prevalent in sexual networks with behavioral and demographic characteristics leading to higher risk for HIV-1 transmission^{31, 37, 38}.

Our analyses have limitations. First, it is likely that most HIV-1 infected partners in our study had chronic, as opposed to acute, HIV-1 infection. Some have speculated that subtype C is associated with higher viremia during acute infection that may contribute to increased transmission^{39, 40}. However, in a separate analysis of seroconverters from our studies, we found no significant association between subtype C and plasma HIV-1 RNA levels during early HIV-1 infection³⁰. Second, as subtypes are geographically distributed, there may be unmeasured differences across study sites that could potentially confound the results, in spite of our assessment of a number of behavioral, demographic, and clinical factors for potential confounding. In the primary cohorts from which our case-control sample derived, there was higher incidence of HIV-1 transmissions within couples in southern Africa (3.7 per 100 person-years, 95% CI 2.6–4.8) compared to couples in eastern African (2.2 per 100 person-years, 95% CI 1.7–2.7), a difference that was statistically significant in a proportional hazards model adjusted for age, gender, circumcision status and unprotected sex (adjusted HR 1.65, 95% CI 1.14–2.38, $p=0.007$); however, our results suggest that this difference is not explained by subtype. The selection of controls from our analysis was based on gender and geographic distribution of the primary cohort to ensure a representative population of controls from the entire cohort.

In summary, we found no statistically significant differences in risk of heterosexual HIV-1 transmission associated with HIV-1 subtype C infection, nor was subtype C significantly associated with higher HIV-1 plasma and genital concentrations. A better understanding the impact of viral diversity on HIV-1 transmission and pathogenicity is important to HIV-1 prevention efforts, including treatment and vaccine development. The role of subtype on HIV-1 disease progression and pathogenicity should continue to be evaluated, particularly to inform the development of a globally applicable cross-protective vaccine.

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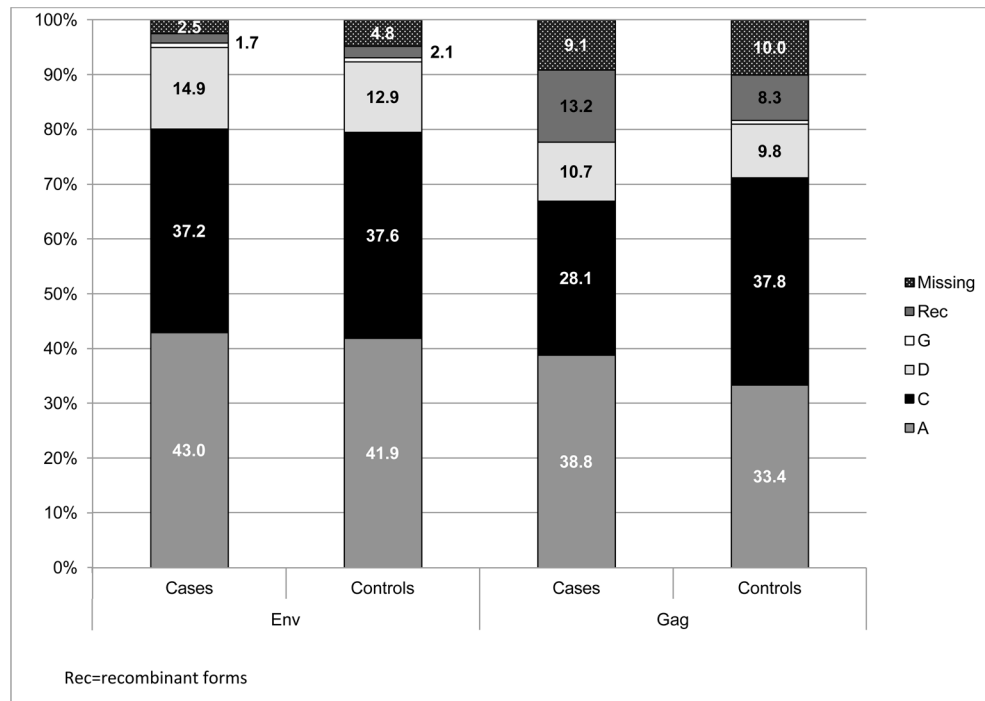


Figure 1. Distribution of *env* and *gag* subtype among cases and controls

The percentage distribution of HIV-1 subtypes by cases (HIV-1 infected partner in transmitting couples, determined to be linked by viral sequencing) and controls (HIV-1 non-transmitting controls) for both the *env* and *gag* gene regions. Letters refer to the subtype for that gene region (RF=recombinant forms)

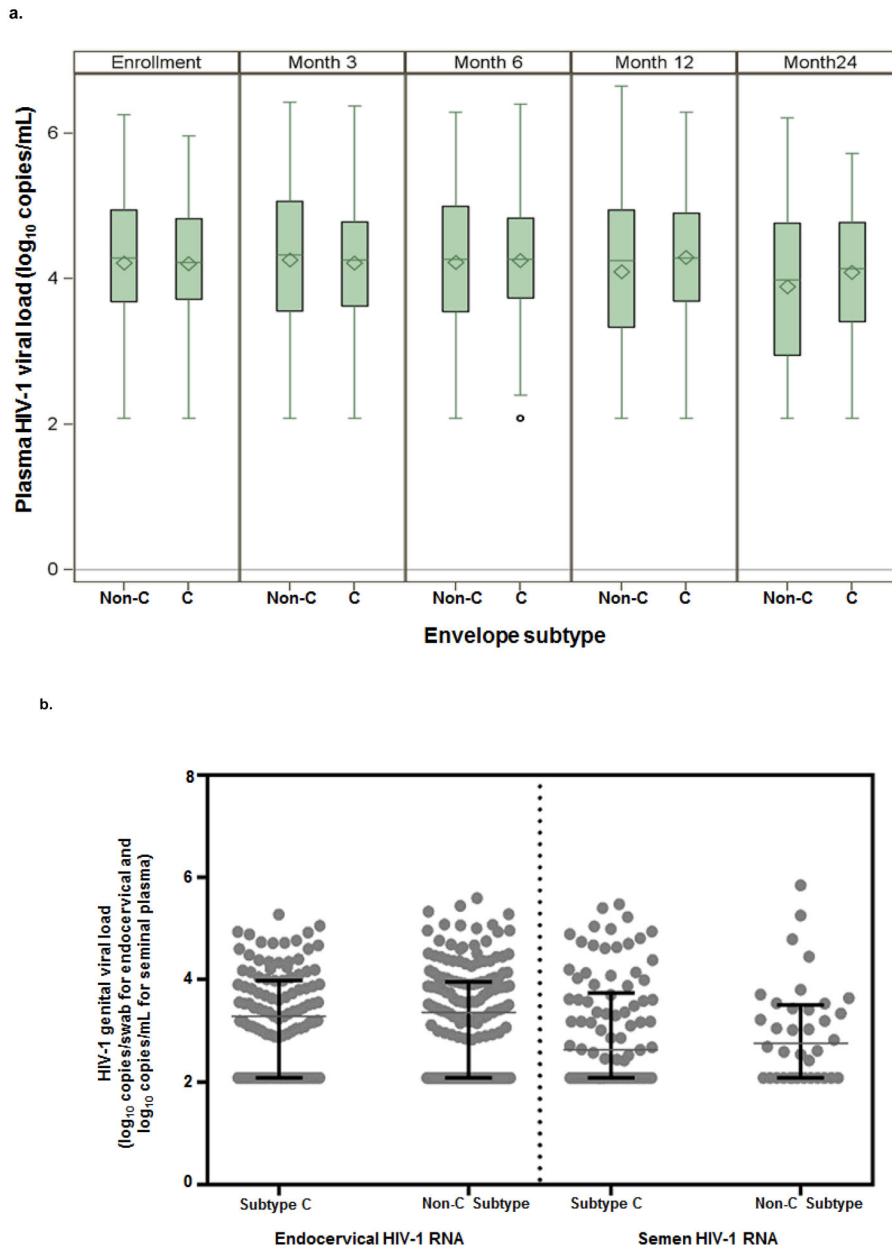


Figure 2. Median plasma and genital HIV-1 RNA by *env* subtype C and non-C subtypes
 a) Box plot distribution of log₁₀ plasma HIV-1 RNA for *env* subtypes C and non-C subtypes by study month. Mean values denoted by diamonds and median values denoted by bars.
 b) Median and interquartile range (IQR) for endocervical and semen HIV-1 RNA concentrations for those with *env* subtype C and non-C subtypes. Individual HIV-1 RNA concentrations plotted with median HIV-1 RNA concentration denoted and IQR denoted by black bars.

Table 1

Characteristics of the study population

	Transmitting couples, N=121		Non-transmitting couples, N=501		Subtype C ^{***} , N=217		Non-C Subtypes ^{***} , N=362	
	N/median	%IQR	N/median	%IQR	N/median	%IQR	N/median	%IQR
<i>Demographic characteristics</i>								
HIV-1 infected female	60	49.6%	328	65.5%	149	68.7%	208	57.5%
Age in years, HIV-1 infected partner	30	26-35	32	26-38	32	27-39	30	26-37
East African (vs. southern African)	80	66.1%	332	66.3%	25	11.5%	353	97.5%
Married/living together	113	93.4%	459	91.6%	179	82.5%	353	97.5%
Duration of partnership, years	3.8	1.5-7.0	5	2.2-9.7	4.1	1.8-8.7	5.2	2.2-9.6
Number of children within partnership	1	0-2	1	0-2	1	0-2	1	0-2
Unprotected sex								
One month prior to enrollment	56	52.8%	160	36.2%	81	37.3%	125	34.5%
Follow-up visits with unprotected sex ^{**}	220/1057	20.8%	643/9280	6.9%	411/3365	12.2%	420/6168	6.8%
Antiretroviral therapy initiated during follow-up	1	0.8%	67	13.4%	6	2.8%	54	14.9%
<i>Baseline clinical characteristics</i>								
CD4 count, cells/mm ³	417	302-580	434	341-601	418	316-570	428	332-602
HIV-1 plasma viral load, log ₁₀ copies/mL	4.8	4.3-5.1	4.2	3.6-4.8	4.2	3.7-4.8	4.4	2.9-4.0
Any genital tract infection (either partner) [*]	23	20.4%	74	15.9%	51	23.5%	43	11.9%
Circumcision (male HIV-1 uninfected partners)	39	65.0%	239	72.9%	84	38.7%	175	48.3%

* Includes *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis*

** Numerator=all followup visits with unprotected sex reported, denominator=total follow-up visits

*** Subtype data shown for *env*; *gag* results were similar (N=43 without subtype information)

IQR=interquartile range

Bold indicates statistical significance at the p<0.05 level, comparing transmitting to non-transmitting couples and subtype C to non-C subtypes

Table 2
Adjusted multivariate models for the nested case-control and sensitivity case-cohort analyses comparing HIV-1 transmission for subtype C versus non-C subtypes

	Nested case-control			Case-Cohort		
	Odds ratio (95%CI) adjusted for gender, age and unprotected sex	Odds ratio (95%CI) adjusted gender, age, unprotected sex <i>plus</i> plasma HIV-1 RNA	Hazard ratio (95%CI) adjusted for gender, age and unprotected sex	Hazard ratio (95%CI) adjusted for age, unprotected sex <i>plus</i> plasma HIV-1 RNA		
	<i>env</i>	<i>gag</i>	<i>env</i>	<i>gag</i>	<i>env</i>	<i>gag</i>
C vs. non-C	1.14 (0.74-1.75) p=0.6	0.98 (0.63-1.52) p=0.9	1.18 (0.76-1.83) p=0.5	1.03 (0.66-1.60) p=0.9	1.56 (0.89-2.76) p=0.1	0.92 (0.51-1.67) p=0.8
C vs. A	1.17 (0.74-1.84) p=0.5	1.09 (0.68-1.75) p=0.7	1.25 (0.78-1.99) p=0.3	1.13 (0.70-1.84) p=0.6	1.22 (0.65-2.29) p=0.5	0.88 (0.45-1.72) p=0.7
C vs. D	1.39 (0.76-2.56) p=0.3	1.79 (0.93-3.47) p=0.08	1.27 (0.68-2.38) p=0.5	1.60 (0.82-3.13) p=0.2	2.19 (0.95-5.06) p=0.07	1.29 (0.49-3.37) p=0.6
					2.29 (0.95-5.51) p=0.07	0.91 (0.45-1.83) p=0.8
						1.28 (0.45-3.62) p=0.6

Adjusted for gender and age of HIV-1 infected partner

Unprotected sex and plasma HIV-1 RNA assessed at baseline in nested case-control model and longitudinally for case-cohort model