Title: Effect of *population viral load* on prospective HIV incidence in a hyper-endemic rural African community

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One Sentence Summary:

Our results demonstrate that HIV *population viral load* indices that explicitly account for the underlying variations in HIV prevalence could play a key role in targeting prevention interventions to vulnerable communities in hyper-endemic African populations.

Abstract

Monitoring HIV *population viral load* (PVL) has been advocated as an important means of inferring HIV transmission potential and predicting the future rate of new HIV infections in a particular community. However, the relationship between the PVL measures and directly-measured HIV incidence has not been quantified in any setting and most importantly not in a hyper-endemic sub-Saharan African setting. We assessed this relationship using one of Africa's largest population-based prospective population cohorts, in rural KwaZulu-Natal, South Africa in which we followed 8,732 HIV-uninfected participants between 2011 and 2015. Despite clear evidence of spatial clustering of high viral loads in some communities, our results demonstrate that PVL metrics that are derived from aggregation of viral load data only from the HIV-positive members of a particular community did not predict HIV incidence in this typical hyper-endemic, rural African population. Only once we used modified PVL measures which combined viral load information with the underlying spatial variation in the proportion of the population infected (HIV prevalence) did we find a consistently strong relationship with future risk of HIV acquisition. For example, every 1% increase in the overall proportion of a population having a detectable virus, was independently associated with a 6.3% increase in the risk of HIV acquisition ($p=0.001$). In hyper-endemic African populations, these modified PVL indices could play a key role in targeting and monitoring interventions in the most vulnerable communities where the future rate of new HIV infections is likely to be highest.

Introduction

The dramatic scale-up of combination antiretroviral therapy (ART) to over 15 million people with HIV(*1*) has resulted in substantial population-level reductions in HIV-related mortality as well as evidence of reductions in the rate of new HIV infections in some developed(*2*) and developing country contexts (*3*). Notwithstanding this impact, the rate of new HIV infections remains unacceptably high with 70% of new HIV infections continuing to occur in sub-Saharan Africa (*4*). Some mathematical models suggest that the epidemic could be reversed by 2050 if high-levels of ART coverage are achieved (in combination with other effective interventions such as medical male circumcision). Scaling up ART therefore remains a key global priority. In response, the UNAIDS has set "the ambitious but achievable" targets of diagnosing 90% of all people living with HIV, to initiating 90% of all diagnosed people on ART, and achieving virologic suppression for 90% of people on ART by 2020; with the aim of increasing all of these targets to 95% by 2030 (*5*). As the world ramps up to meet and exceed these targets, it becomes critical to not only monitor progress, but also provide empirical evidence for any resultant impact of expanding levels of viral suppression on life expectancy and the rate of new HIV infections at a population level.

One of the ways in which the impact of moving towards the UNAIDS 90-90-90 treatment targets could be monitored is through measuring the trends in HIV *population viral load* (PVL) (*6, 7*). The HIV viral load level in semen or blood is the single most important biological determinant of transmission between an HIV-positive and an HIV-negative individual (*8, 9*). It follows that an aggregation of individual HIV viral RNA concentrations for a particular geography or community over a given time period (*viz,* PVL) could constitute a sensitive biological index of treatment programme success as well as potentially estimate the

"transmission potential" of a particular geography. Indeed, *community viral load* has been endorsed as a key measure by the CDC(*10*), and has been used to infer both the effectiveness of a treatment programme as well as a proxy for HIV incidence (*2*, *11*-*13*).

Notwithstanding the above, the PVL concept as a measure of both transmission potential and ART programme effectiveness has been critiqued (*14*). One key issue raised by the authors is the degree to which the in-care or facility-based viral load measures are reflective of the true underlying population viral load. Other issues raised include the interpretation of various population viral load metrics and their relationship to ongoing HIV transmissions, ecologic bias due to aggregation over large geographic regions or heterogeneous populations, and the failure of some PVL measures to account for the underlying HIV prevalence within the community. To date no study has tested the relationship between these PVL measures and directly-measured HIV incidence in a hyper-endemic sub-Saharan African setting.

Here we make use of one of Africa's largest population-based prospective cohorts, which is located in the rural KwaZulu-Natal province of South Africa, to empirically quantify the relationship between the true population viral load (among all HIV-infected participants irrespective of whether these participants have accessed care or know their HIV status) and the prospective risk of HIV acquisition for over 8,700 participants who were HIV-uninfected at baseline (HIV incidence). We use novel geospatial techniques to analyse the micro-level spatial variation in three key PVL measures—called the *geometric mean viral load* (MVL), *prevalence of detectable viremia* (PDV), and the *community transmission index* (CTI)—derived from all HIV-positive participants. We then extend these three measures to consider not just HIV positive participants but to evaluate the entire population (irrespective of HIV status).

Results

Population-based viral load survey

This study uses data from one of the most comprehensive demographic and HIV surveillance sites in Africa—the Africa Centre Demographic Information System.(*15*) The site has collected socio-demographic information on a population of 87,000 participants within a circumscribed geographic area (438 km^2) for over a decade. Within the study site, ongoing population-based HIV surveillance and sexual behaviour surveys take place annually among all adults ≥15 years of age. Of those contacted during the 2011 survey, 78.6% agreed to be tested in the survey and provide a dried blood spot (DBS) sample.(*3*) We performed viral load measurements on the blood-spots of all 2,456 participants who tested HIV-positive in the population-based HIV surveillance round of 2011. Using the Generic HIV Viral Load kit (Biocentric), we successfully obtained 2,420 (98%) viral load measurements after nucleic acid extraction with NucliSENS® EayMag® (Bordeaux, France). The methodology for quantifying the HIV-1 RNA viral loads has a lower limit of detection of 1550 copies per mL and is described in greater detail elsewhere.(*16*)

The geometric mean population-based viral load (95% CI) among HIV-infected people was 8,259 (7,507–9,087) copies/mL (Table 1). Of the 2,420 HIV-positive participants in the population-based cohort, 30% had undetectable viral loads (n=726) and 26% (n=629) had a viral load >50,000 copies/mL. Males had overall higher geometric mean viral loads and a higher proportion of males were characterised by viral loads of >50,000 copies/ml (Figures 1A, 1B).

Spatial variation in population viral load indices

We used the viral load measurements of all HIV-positive participants (N = 2,420) from the 2011 survey to construct the following PVL indices across the study area: 1) The *mean viral load* (MVL), which is the geometric mean of the viral loads among all HIV-positive participants in the population-based survey. 2) The *prevalence of detectable viremia* (PDV), which represents the proportion of the HIV-positive population that have a detectable viral load (>1550 copies/mL).(*16*) 3) The *Community Transmission Index* (CTI), which is a potentially more sensitive biological measure than 1) and 2), and represents the relation between the viral load and the risk of HIV transmission per unprotected sexual contact.(*17*) To derive this measure, we used the result from Quinn et al.(*8*), who showed that each $\log_{10}$ increment in viral load was associated with 2.45 fold increase in the rate ratio for HIV transmission risk. Following the notation of Wilson et al.(*17*), the risk of HIV transmission per sexual act is therefore given by $\beta_1 = 2.45^{\log_{10}(V_1/V_0)}\beta_0$, where V_1 is the viral load level associated with the participant, $V_0 = \log_{10}(150)$ is a baseline viral load level, and $\beta_0 = 0.003$ is the probability of HIV transmission from a person with the baseline viral load level (V_0).(*17*) The β_0 value is based on prior research undertaken in the sub-Saharan African context.(*18*) Using the standard binomial formula, we then calculated $CTI = \left(1 - \left[1 - \beta_1\right]^{100}\right) \times 100$ to obtain the estimated number of transmission events that occur in 100 sexual contacts between persons at risk of infection and HIV-positive members of a particular community. We standardised all of the PVL measures against the age (10-year bands) and sex characteristics of the eligible population in 2011.

Next, we constructed population-based versions of the three PVL indices described above based on the population in each community irrespective of HIV-status. In effect, all HIV-negative participants in a particular community are included by assigning a viral load value of

zero (to construct the MVL_P[*] and PDV_P indices) or a transmission probability of zero (to construct the CTI_P index). Thus for these analyses, we included 7,919 individuals who tested HIV-negative in 2011 (total tested = 10,375). Hence, the denominator of these modified indices becomes the entire population rather than only those who have tested HIV-positive. By including information on the number of HIV-negative participants in a particular community, these modified PVL measures inherently account for the underlying spatial variation in HIV prevalence.

Marked spatial heterogeneity in all of the PVL measures were observed across the surveillance area. There was clear evidence of spatial clustering of participants with high geometric mean viral loads (Figure 2A), participants with detectable viral loads (Figure 2B) and estimated number of transmission events per 100 sexual contacts between individuals at risk of infection and HIV-infected members of a particular community (Figure 2C). Likewise, clear evidence for spatial clustering of these modified PVL measures were observed when we considered the entire adult population (Figure 2D – F) in the calculation. Remarkably, in some of the high-incidence communities, whilst 65% of the HIV positive individuals had unsuppressed viral loads (Figure 2B), > 20% of the entire population (irrespective of HIV status - i.e. PDV_P) were viremic for HIV (Figure 2E), underscoring the scale of the epidemic in these areas.

Relationship between the population viral load measures and the risk of HIV acquisition

We followed up all 8,732 repeat-testers who were resident within the surveillance area between January 2011 and December 2015. During this period we observed 859 sero-

[*] We use the subscript $_P$ to denote the fact that these modified versions of the PVL indices utilize information on the full population (irrespective of HIV-status) rather than only including those who have tested HIV-positive

conversions and 26,219 person-years of observation in the 8,732 repeat-testers who were HIV uninfected at baseline (crude incidence = 3.28 events per 100 person-years [Table 2]). Our aim was to quantify the relationship between the PVL measures which are derived from the HIV positive population and the prospective risk of HIV acquisition using a Cox proportional hazards model. We then compared these results against the set of PVL indices derived from the entire adult population (irrespective of HIV status).

At an ecological level, communities with the overall highest viral loads and highest levels of unsuppressed viral loads were not characterised by the highest prospective crude HIV incidence (Figure 3 A-C). However, with respect to the measures constructed on the basis of those who were HIV-infected as well as those who were HIV-uninfected, there was a clear dose-response pattern evident with communities with the highest overall viral loads across the whole population (irrespective of HIV status) having the highest crude HIV incidence (Figure 3 D-F).

Results for the individual-level Cox proportional hazard models demonstrated that none of PVL measures derived from the HIV-positive population predicted individual HIV acquisition risk, both before and after controlling for key socio-economic and behavioural factors as well as HIV prevalence in the surrounding local community (aHR=1.000–1.048, p-values ranged from 0.057–0.97) as shown in Table 3 (Models A1–A3). Addition of the PVL indices to the base model did not improve the model fit, as determined by the Akaike information criterion.

By contrast, our results show that all of the modified PVL indices based on the entire adult population (irrespective of HIV status) were all highly predictive of future HIV acquisition risk both before and after controlling for key socio-economic and behavioural factors and were robust to the addition of HIV prevalence in the model (Models B1–B3 of Table 3). A one unit increase in geometric mean population viral load (MVL_P) was independently associated with a

9.1% increase in the risk of HIV acquisition, (aHR=1.091, p<0.001; Model B3 of Table 3). Every 1% increase in the population prevalence of detectable viremia (PDV_P) was independently associated with a 6.3% increase in the risk of a HIV acquisition (aHR= 1.063, p=0.001; Model B3 of Table 3). Finally, every additional unit increase in the community transmission index (CTI_P) was independently associated with a 19.3% increase in the risk of HIV acquisition, (aHR= 1.193, p=0.001; Model B3 of Table 3).

HIV prevalence in the surrounding local community was a highly significant predictor of risk of new infection before and after controlling for other multiple independent risk factors (Table S2, Supplementary Materials). However, as expected the HIV prevalence effect was rendered insignificant by the addition of any of the modified PVL metrics that were calculated on the entire population irrespective of HIV-status (Table S5, Supplementary Materials). This is because these modified PVL indices already inherently account for variations in HIV prevalence as they incorporate information on the spatial distribution of both HIV-negative and HIV-positive cases. In line with our previous work, across all the models, being older (>35 years), reporting no sexual partners in the last 12 months (versus one or more), and living in a household with higher wealth was protective against HIV acquisition (3).

Sex-specific population viral load patterns and risk of acquisition of infection in the opposite sex

As a further robustness check of our findings, we constructed sex-specific PVL indices in the same manner. We then conducted a set of parallel analyses to ascertain the degree to which a female's HIV acquisition hazard was related to viral load patterns (and HIV prevalence) of men in the surrounding local community and *vice-versa*. The ability to detect any PVL effect is markedly attenuated in these analyses (due to smaller overall observation time and numbers of

events) and the fact that the PVL measures are subject to more random noise given the smaller number of observations used in their construction. Nevertheless, this suite of analyses also confirmed the same pattern described in the mixed sex analyses above. In these models, the PVL measures of the HIV-positive population was similarly not associated with HIV acquisition risk in the opposite sex both before and after adjustment for key socio-economic, behavioural risk factors as well as HIV prevalence in the surrounding local community (p-values ranged from 0.18 to 0.99; Table 4A and 4B). By contrast, all of the modified PVL measures which included information on the HIV negative population were strongly predictive of acquisition risk in the opposite sex (p-values ranged from <0.001 to 0.048).

Population viral load measures derived from routine data collected at health facilities

Since facility-based viral load data are typically used to derive community viral load metrics in many settings, we also wanted to establish the degree to which facility-based viral load measurements corresponded with their population-based counterparts and whether such data could be harnessed to infer transmission potential. We used the viral load measurements from routine clinical data collected at the facility level for 3,196 patients living in the surveillance population who visited one of the 17 health-care clinics in 2011 to compute the facility-based VL versions of the measures described above (*19*). We were able to link these patients to the Africa Centre population database. All participants from the population- and facility-based viral load surveys were geo-located to their exact homestead of residence (*3*).

Results show that the overall geometric mean (95% CI) viral load for the facility-based data was 819 (771–870) copies/mL (Table S1 of the Supplement). Of the 3,196 HIV-positive participants in the facility-based cohort, 4% (128) had a viral load >50,000 copies/mL. Males

had overall higher geometric mean viral loads and a higher proportion of males were characterised by viral loads of >50,000 copies/ml (Figure S1 of the Supplement).

Use of routine facility-based viral load data (*19*) to construct the viral load indices, demonstrated that there was little or no correlation between these indices and those derived using the equivalent population-based viral load data (correlation coefficient = –0.089, –0.32 and –0.28 for MVL, PDV and CTI respectively). Empirically, there was no relationship between any of the facility-based viral load measures and future risk of acquisition of HIV infection both before and after adjustment for key risk factors as well as HIV prevalence in the surrounding local community (Table S8 of the Supplementary Materials).

Discussion

Our study has empirically tested the relationship between various population viral load metrics and the risk of HIV acquisition in a typical hyper-endemic rural African setting. Despite identifying remarkable spatial variation in viral load patterns across the study area (with clear evidence of clustering of high viral loads in some communities), we found no relationship between any of the PVL indices derived solely from HIV-infected participants and HIV incidence. Our findings occurred in the context of using population-based viral load measurements that were largely free from the biases typically associated with health facility-based viral load data. Only when we evaluated the viral load information as a function of the entire population (both HIV-positive and HIV-negative participants), were the resulting measures highly predictive of new HIV infections. This pattern also held in a series of parallel analyses in which we tested the relationship between the sex-specific population viral load patterns and the HIV acquisition risk in the opposite sex. Furthermore, our results demonstrate that in this rural African setting, facility-based viral load measures do not correlate well with their population-based counterparts and were not predictive of HIV incidence. On the basis of these findings we would therefore caution against using such data to infer transmission potential in similar settings.

Another important finding to emerge from the research is large differences in HIV viral load by sex at a population level. Overall males had a higher geometric mean viral load in comparison to females. For example, the geometric mean viral load of males in the 20–24 year age group was 1.21 times higher than in the corresponding female age group. This finding likely reflects the fact that men are less likely to diagnose HIV positive and successfully link to care, as

well as being less likely to successfully adhere to treatment supporting earlier findings in this setting as well as others (*20-22*).

Community viral load has been viewed a proxy for HIV transmission potential and/or programme effectiveness in many developed (*2, 11, 23, 24*) and developing country contexts (*13*). However, such studies are based on ecological (correlations between group-level variables and group-level out-comes) and as such provide a weak basis for causal inference (*25*). Most recently, Solomon and colleagues conducted a cross-sectional, study in India which estimated the site-level correlations between four CVL measures and an estimate of HIV incidence (derived from a multi-assay algorithm) in people who inject drugs and men who have sex with men (*13*). In this high-risk population grouping, the authors demonstrate site-level correlations between three community viral load measures and the assay-derived estimate of HIV incidence. The "prevalence of viremia," measure (defined as the local prevalence of HIV-infected individuals with HIV viral loads >150 copies/mL) showed the strongest correlation to the incidence estimate. Similar to the findings in this study, the "in-care viral load" measure was not correlated with the HIV incidence estimate among the high-risk groups, despite being a commonly used community viral load measure (*2*) (*26*).

Existing methods of calculating the community viral load have been critiqued for a number of reasons related to: 1) the use of non-representative samples in facility-based (or in-care) data, 2) the failure to incorporate information on population HIV prevalence, and 3) improper aggregation across wide geographic regions or heterogeneous populations that make ecological biases unavoidable.(*14*) We constructed PVL measures that address criticism 1) by using information from all HIV-infected participants from a full population cohort (irrespective of their linkage to care or knowledge of HIV status) and address criticism 2) by modifying the

recommended PVL measures to incorporate information on the underlying variations in HIV prevalence (by including information on the distribution of HIV sero-negative individuals in the derivation of the PVL indices). We have addressed criticism 3) by creating a sensitive and relevant PVL estimate derived from the unique community around each HIV-uninfected member of the population-based, sero-incidence cohort and directly measuring the time to HIV sero-conversion in each individual in the cohort. Using this approach, we were able to control for a wide range of individual-level confounders in the analysis and avoid ecological fallacies in interpretation of the results.

Nevertheless, our work has some limitations. Most notably, the link between the PVL in the surrounding local population and HIV acquisition risk is dependent on a reasonable proportion of the population preferentially selecting partners from the surrounding local community. However, if this were not the case, we would not expect any of the community-level PVL indices used in the analyses to significantly influence risk of HIV acquisition. As indicated above, we find strong independent relationships between all of the modified PVL measures that analyse the entire population (irrespective of HIV status) and risk of HIV acquisition. Moreover, in our previous work we reported that partner choice is strongly affected by geography with 61% of women reporting at least one partnership with a man in the same immediate local Zulu community over a 5-year period (*27*). A potential second limitation is that we treat the PVL exposure as being time-invariant. Whilst unlikely, it is theoretically possible that there could be large systematic geographic shifts in viral load patterns over time that could bias the result (particularly in a more sensitive PVL measure such as the *CTI*). However, such a "measurement error" would likely bias the finding towards the null hypothesis and would not account for the

significant positive associations identified between both community-level HIV prevalence as well as all of the modified PVL measures and future risk of HIV acquisition.

In our previous work we demonstrated substantial space-time differences in ART coverage in this population as the ART programme had scaled up over eight years (2004-2011) and we found that these large differences in ART coverage independently predicted individual HIV acquisition risk (*3*). By 2011, the heterogeneity in ART coverage had decreased because the scale-up of ART had been so rapid in all parts of the study area. In 2007, for example, only 6.4% of the population lived in communities where ART coverage exceeded 30%, and by the middle of 2011 this figure had increased to 90.8% (*3*). Population viral load might be considered a more proximate measure of transmission potential and therefore one might expect that any of the PVL measures would be a stronger predictor of HIV acquisition risk than ART coverage alone. Aside from highlighting the importance of accounting for underlying HIV prevalence in the population, this null finding also illustrates the difficulty of extrapolating the biological differences seen within-host to the population in a meaningful way. Such difficulties are particularly salient for sexually transmitted infections which are dependent on highly selective sexual partnership patterns including the phenomenon of HIV "sero-sorting" (*28*).

Thus, large differences in viral load patterns between two populations would be relatively meaningless from a transmission potential perspective if there is a high degree of sero-sorting in the populations or if high viral loads occurred mainly in the segment of the population that were no longer sexually active as a result of HIV-related illness. Further, there are other possible pathways through which ART coverage might reduce the risk of new HIV infection that are in addition to the biological effect of reducing viral load and consequently HIV transmission potential (*3*). For example, a community with high levels of ART coverage, would necessarily

have had high levels of exposure to HIV counselling. This in turn, could lead to changes in sexual behaviour in both HIV-uninfected and HIV-infected individuals.

There has been much discussion and debate about the utility of PVL measures to infer HIV transmission potential (*13, 14, 29*). Our results demonstrate that HIV *population viral load* indices that explicitly account for variations in the relative size of the HIV uninfected population could play a key role in targeting prevention interventions to vulnerable communities in hyper-endemic African populations. Recent work has revealed the existence of sub-epidemics in many generalized epidemic settings providing a clear rationale for targeting areas of high transmission (*30*). There has also been a recent shift towards a more locally-tailored epidemic response on the basis of differences in both the underlying epidemiology and characteristics of the population-at-risk (*31-33*). In this regard, our findings show that even in a severely affected rural African population with a well-established HIV treatment programme, a PVL measure such as the proportion of the overall population having HIV viremia could play a role in targeting and monitoring the effectiveness of interventions in the most vulnerable communities where future levels of HIV incidence are likely to be highest.

Materials and Methods

Population-based HIV survey

Since 2004, the Africa Health Research Institute has conducted annual population-based HIV testing of all consenting adults aged 15 years or older (*15*). After obtaining written informed consent, field workers collect blood by finger prick and prepare dried blood spots for HIV testing according to the UNAIDS and WHO *Guidelines for Using HIV Testing Technologies in Surveillance*. Overall 29% of the adult population are infected with HIV (*34*). The rate of new infections remains high and relatively constant over time at around 3 new infections per 100 person-years (*3*, *35*). The incidence of new HIV infection peaks in women at approximately 7.5 per 100 person-years (at age 25) and in men at 5 per 100 person-years (at age 30). The population is characterised by low levels of marriage with only 31% of women and 23% of men ever having been married and 14% of those marriages being polygamous for men (*36*).

Construction of the PVL measures

We used the viral load measurements of the 2011 population-based survey to obtain sensitive and realistic PVL measures in the unique virtual community surrounding each homestead in the study area. These PVL measures were computed by means of a moving two-dimensional Gaussian kernel of 3 km search radius (*37*). The size of the kernel was determined from the results of previous work (*38*). First, all participants were located to an exact homestead of residence and the viral load measurements are superimposed on a geographic representation of the study area consisting of a grid of 30m x 30m pixels. Next, the kernel moves systematically across the grid and calculates a Gaussian weighted estimate of the PVL measure for the unique

neighbourhood around each and every pixel on the grid. The method is well suited to the scattered distribution of the population because it does not impose any static geographical boundaries on the data. Instead, it uses the precise location of each homestead to derive a PVL measure that is both responsive to local variations and robust to the effects of random noise. We used exactly the same methods to quantify spatial variations in the viral load indices obtained from routine clinical data. These data were collected at the facility level for 3,196 patients living in the surveillance population who visited one of the 17 health-care clinics in the sub-district in 2011. Patients were geo-located to their exact homestead of residence through linkage to the population database (*19*).

Statistical analysis

We followed up all 8,732 repeat-testers who were resident within the surveillance area between January 2011 and December 2015. A repeat-tester is a study participant who was known to be HIV-negative on the 1st Jan 2011 and who was tested for HIV during the study period. Because the data are interval censored, we imputed a single random sero-conversion date (using a uniform distribution) between the repeat-tester's latest HIV-negative and earliest HIV-positive dates as described in greater detail elsewhere.(*39*) We evaluated each of the PVL measures separately in the analysis. We adjusted for well-established determinants of HIV acquisition identified in our previous work (*3, 35*); that is sex, age, area of residence (rural/urban), marital status, number of partners in the last 12 months, household socio-economic status (based on household assets) and HIV prevalence in the unique community surrounding each HIV-negative individual. We computed community-level HIV prevalence using the Gaussian kernel methodology described above based on data from 10,375 participants who participated in

population-based HIV testing in 2011 (*3*). We treated the PVL measures and the covariates as time-invariant. In other words, a repeat-tester was exposed to the PVL of his or her surrounding local community for the duration of the study (i.e., until the right censoring date).

Secondly, we investigated the relationship between the PVL measures based on the entire population (irrespective of HIV status) and future risk of acquisition of HIV acquisition to an HIV-uninfected individual using the same methodology. Thirdly, as a further robustness check of our findings we conducted a series of analyses to test whether the same patterns could be seen in the relationship between sex-specific population viral load patterns and risk of acquisition of infection in the opposite sex. Lastly, we performed a further set of parallel analyses to ascertain whether there was any relationship between the community viral load indices derived using the routine facility-based viral load data and future risk of HIV acquisition.

Supplementary Materials

Materials and Methods

Fig. S1. Proportion of facility-based viral loads >50,000 copies/mL by sex for the year 2011.

Table S1. Summary statistics: viral load measurements by sex and age for the routine facility-based survey data.

Table S2. Results of the multivariable analysis (Cox Proportional Hazard model) to examine the relationship between risk of HIV acquisition and three population viral load (PVL) measures constructed from the HIV-positive cases of a population-based survey.

Table S3. Results of the multivariable analysis (Cox Proportional Hazard model) to examine the relationship between risk of HIV acquisition and three community viral load (PVL) measures constructed from the HIV-positive and HIV-negative cases of a population-based survey.

Table S4: Results of the multivariable analysis (Cox Proportional Hazard model) to examine the relationship between the risk of HIV acquisition for females and the three population viral load (PVL) measures. The PVL measures are derived from the male HIV-positive cases of a population-based survey.

Table S5. Results of the multivariable analysis (Cox Proportional Hazard model) to examine the relationship between the risk of HIV acquisition for females and the three population viral load (PVL) measures. The PVL measures are constructed from the male HIV-positive and HIV-negative cases of a population-based survey.

Table S6: Results of the multivariable analysis (Cox Proportional Hazard model) to examine the relationship between the risk of HIV acquisition for males and the three population viral load

(PVL) measures. The PVL measures are derived from the female HIV-positive cases of a population-based survey.

Table S7. Results of the multivariable analysis (Cox Proportional Hazard model) to examine the relationship between the risk of HIV acquisition for males and the three community viral load (PVL) measures. The PVL measures are constructed from the female HIV-positive and HIV-negative cases of a population-based survey.

Table S8. Results of the multivariable analysis (Cox Proportional Hazard model) to examine the relationship between risk of HIV acquisition and three community viral load measures constructed from the routine facility-based survey data.

Table S9. Kuldorff spatial clustering results for the population viral load (PVL) measures displayed in **Fig. 2.**

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Author contributions: FT conceived the paper. AV and FT did the statistical analysis. DC and FT performed the geographical analysis. FT and AV drafted the paper and all authors provided critical input to writing the paper.

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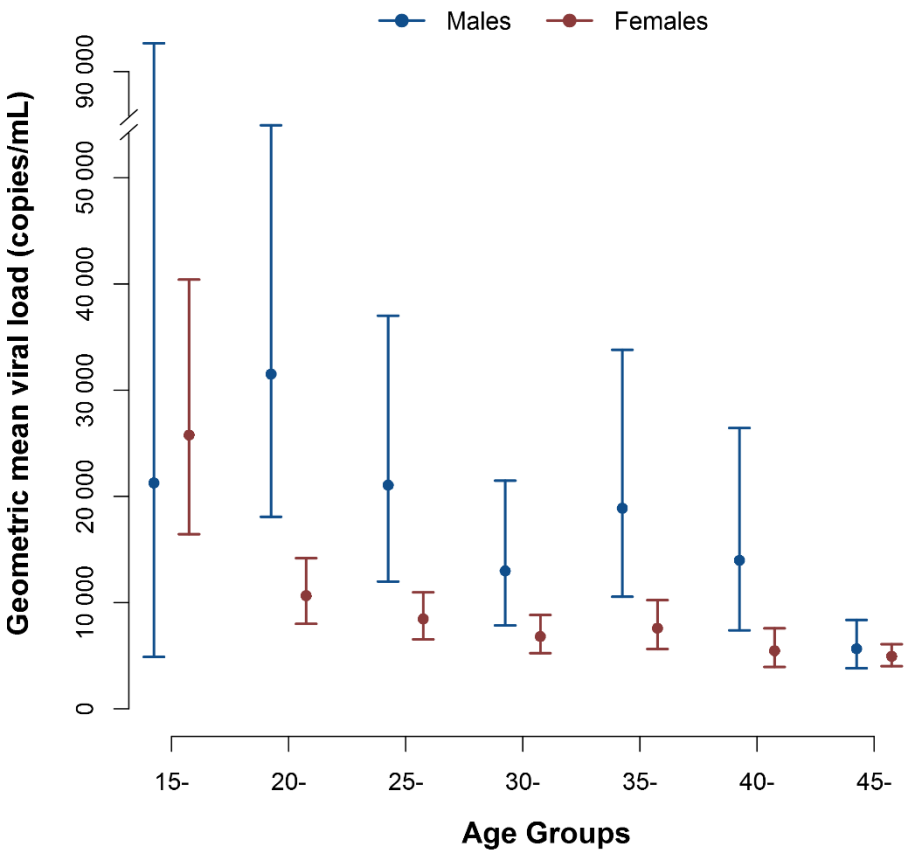
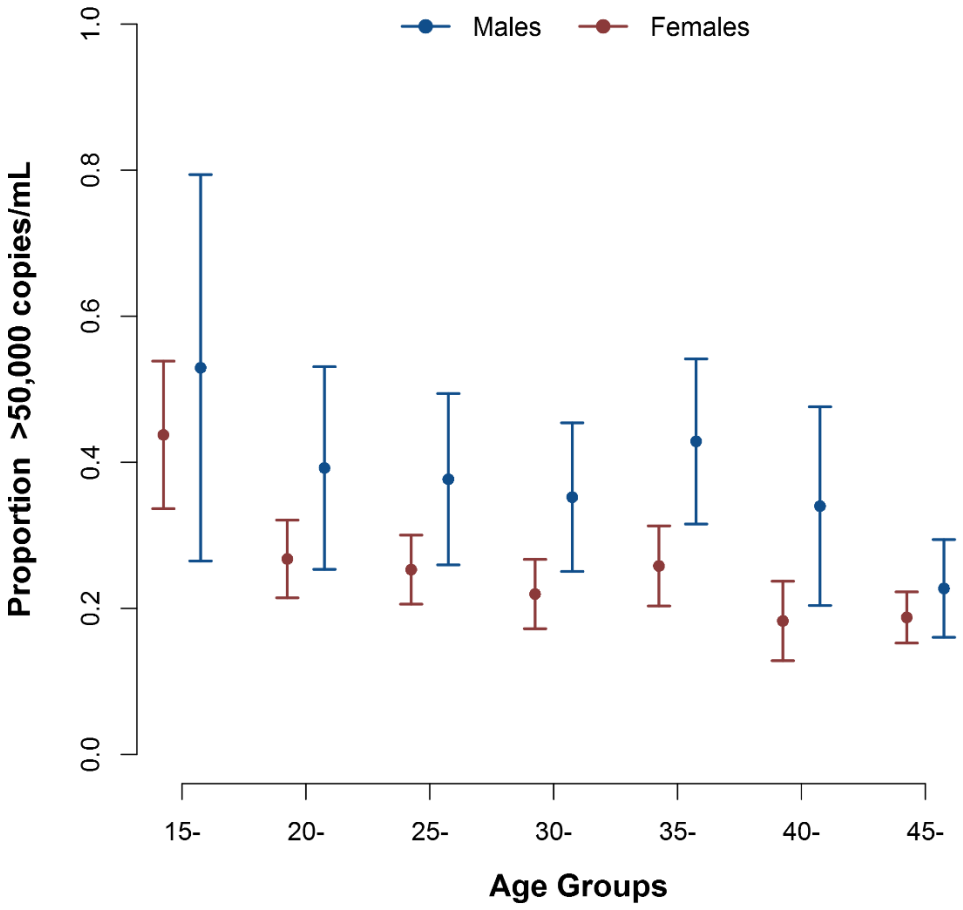
Figures:

Fig. 1A. Geometric mean viral load by sex for the population-based survey (2011).



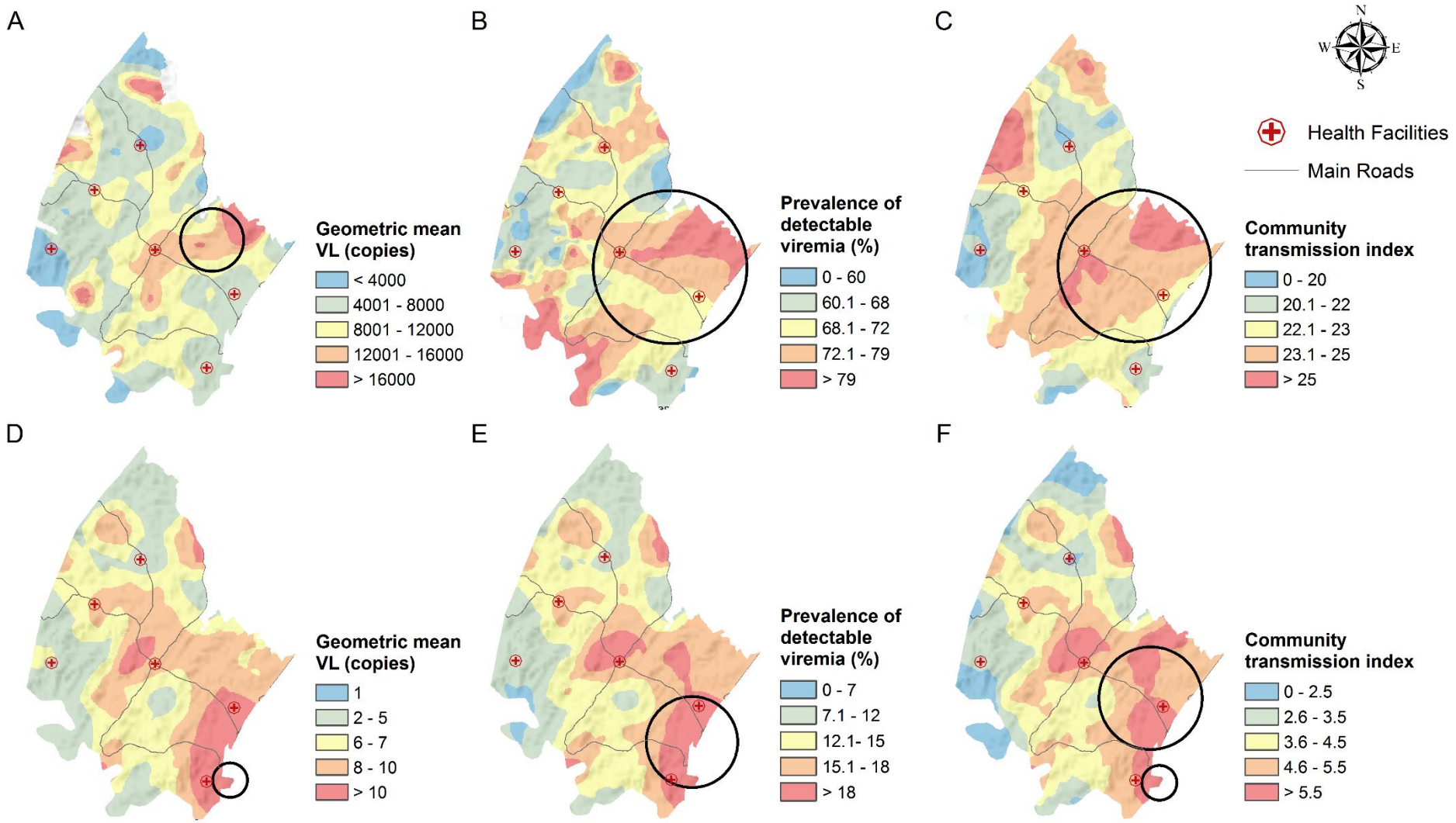
Males older than 20 years of age had higher geometric mean viral loads when compared with females in the same age range. For example, males in the 20–24 and 25–29 age groups respectively had 3.0 and 2.5 times higher mean viral loads than woman in the same age groups. Estimates are shown with their 95% confidence intervals.

Fig. 1B. Proportion of viral loads >50,000 copies/mL by sex for the population-based survey (2011).



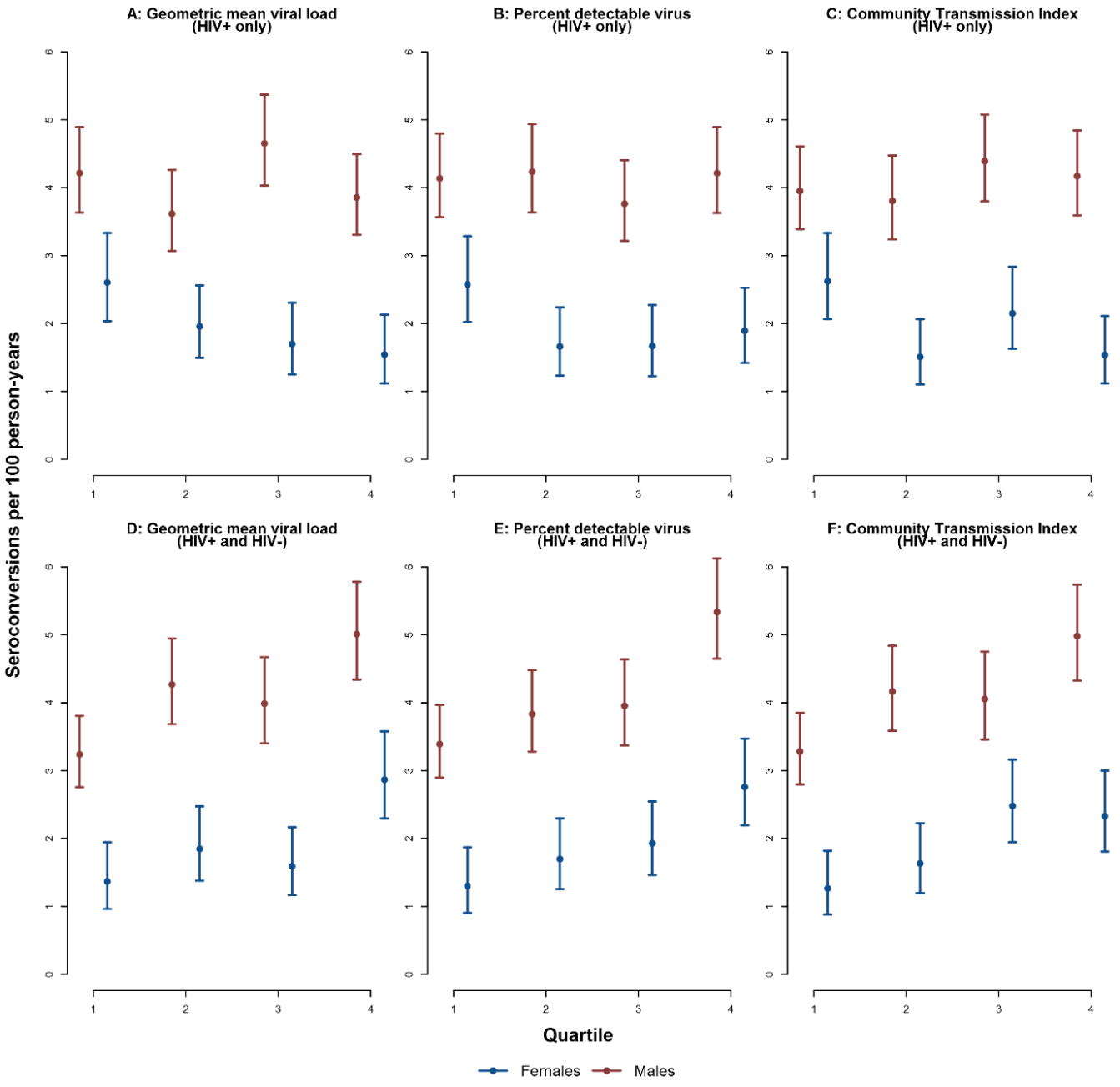
A higher proportion of men had viral loads >50,000 copies/mL when compared with woman (a ratio of 1.2–1.9). Estimates are shown with their 95% confidence intervals.

Fig. 2. Geographical variations in the community viral load indices (HIV+ only; Figures **A-C**), and their population-based equivalents (HIV+ and HIV–; Figures **D-F**) in 2011.



The figure shows the differences in the geographic distribution of the PVL measures when derived from population-based data which excludes (Figures A-C) and includes (Figure D-F) HIV prevalence information. These measures were obtained by using a two-dimensional standard Gaussian kernel of radius 3km. The Kulldorff spatial clusters of high viral loads are shown as black circles.

Fig. 3. Incidence rates (95% CI) by quartile for the community viral load indices (HIV+ only **A to C**) and population-based versions (HIV+ and HIV- **D to F**) by sex for the year 2011.



The figures show the incidence rates per 100 person-years (and interquartile range) for the age-standardised PVL indices by sex for the year 2011.

Tables:

Table 1. Summary statistics: viral load measurements by sex and age for the population-based survey data (2011).

	Samples:		Geometric Mean:		>50,000 copies/mL:	
	N	**(%)**	**copies/mL**	**(95% CI)**	**Proportion**	**(95% CI)**
Overall	2420		8259	(7507–9087)	0.26	(0.24–0.27)
Male	506	(20.91)	12802	(10352–15832)	0.34	(0.3–0.38)
Female	1,914	(79.09)	7356	(6614–8181)	0.24	(0.22–0.26)
Age Group						
15–	98	(4.05)	25139	(15934–39660)	0.46	(0.36–0.56)
20–	295	(12.19)	13508	(10376–17586)	0.29	(0.24–0.35)
25–	405	(16.74)	10815	(8606–13592)	0.28	(0.24–0.33)
30–	360	(14.88)	7766	(6026–10008)	0.26	(0.22–0.31)
35–	353	(14.59)	8460	(6529–10963)	0.28	(0.23–0.32)
40–	243	(10.04)	6823	(5026–9262)	0.23	(0.18–0.29)
45+	666	(27.52)	5238	(4397–6240)	0.19	(0.16–0.22)

Table 2. Sero-incidence rates per 100 person-years for the HIV cohort of repeat-testers who were HIV-uninfected at baseline (2011).

	Person-years	Events	Incidence per 100 person-years	
			Rate	95% CI
Population HIV prevalence				
0–14.9%	935	18	1.92	(1.21-3.05)
15–24.9%	15693	480	3.06	(2.8-3.34)
25+ %	9590	361	3.76	(3.4-4.17)
Sex				
Male	9943	194	1.95	(1.69-2.25)
Female	16276	665	4.09	(3.79-4.41)
Age strata				
15–	9228	257	2.78	(2.46-3.15)
20–	4998	287	5.74	(5.11-6.45)
25–	2838	157	5.53	(4.73-6.47)
30–	1727	66	3.82	(3-4.86)
35–	1735	29	1.67	(1.16-2.4)
40–	2015	30	1.49	(1.04-2.13)
45+	3675	33	0.9	(0.64-1.26)
Area of residence				
Rural	16817	510	3.03	(2.78-3.31)
Peri-urban	8381	312	3.72	(3.33-4.16)
Urban	1019	37	3.63	(2.63-5.01)
Marital status				
Single	15078	448	2.97	(2.71-3.26)
Married monogamous	5898	196	3.32	(2.89-3.82)
Married polygamous	5242	215	4.1	(3.59-4.69)
Number of partners in the last 12 months				
Zero	11827	328	2.77	(2.49-3.09)
One	10291	368	3.58	(3.23-3.96)
More than one	4100	163	3.98	(3.41-4.64)
Household wealth quintile				
Poorest	5348	201	3.76	(3.27-4.31)
2nd poorest	5428	163	3.00	(2.58-3.5)
3rd poorest	5932	218	3.67	(3.22-4.2)
4th poorest	5363	172	3.21	(2.76-3.72)
Wealthiest	4146	105	2.53	(2.09-3.07)
N = 8,732				

Table 3. Results of the multivariable analysis (Cox Proportional Hazard model) to examine the relationship between the risk of HIV acquisition and three community viral load (PVL) measures. The PVL measures were derived from the HIV-positive cases (Models A) and the HIV-positive and HIV-negative cases (Models B) of a population-based survey. The full output is given in Tables S2 and S3 of the supplementary materials.

	Geometric mean viral load*			**Percentage detectable viremia†**			**Community transmission index‡**		
	HR	(95% CI)	p-value	HR	(95% CI)	p-value	HR	(95% CI)	p-value
Population-based: HIV-positive cases only									
Model A1: Unadjusted HR	1.000	(1.000-1.000)	0.254	0.997	(0.987-1.007)	0.503	0.999	(0.957-1.043)	0.966
Model A2: Adjusted HR without HIV prevalence	1.000	(1.000-1.000)	0.817	1.005	(0.994-1.015)	0.401	1.037	(0.990-1.086)	0.130
Model A3: Adjusted HR with HIV prevalence	1.000	(1.000-1.000)	0.475	1.008	(0.996-1.019)	0.190	1.048	(0.999-1.100)	0.057
Population-based: HIV-positive and HIV-negative cases									
Model B1: Unadjusted HR	1.049	(1.029-1.069)	<0.001	1.053	(1.030-1.078)	<0.001	1.187	(1.103-1.277)	<0.001
Model B2: Adjusted HR without HIV prevalence	1.079	(1.046-1.113)	<0.001	1.070	(1.039-1.103)	<0.001	1.224	(1.121-1.337)	<0.001
Model B3: Adjusted HR with HIV prevalence	1.091	(1.045-1.138)	<0.001	1.063	(1.025-1.103)	0.001	1.193	(1.079-1.320)	0.001
N	8732			8732			8732		

Exponentiated coefficients. *For a one unit increase in geometric mean viral load. †For a one percent increase in the prevalence of detectable viremia. ‡For a one transmission event increase per 100 sexual contacts. Model 1 shows the unadjusted Hazard Ratios (HR) for the PVL measures; Model 2 shows these HRs after adjusting for age, sex, urban status, marital status, number of sexual partners in the last year, and household wealth; Model 3 shows these HRs after adjusting for the Model 2 covariates and HIV prevalence.

Table 4A: Results of the multivariable analysis (Cox Proportional Hazard model) to examine the relationship between the risk of HIV acquisition for females and the three male community viral load (PVL) measures. The PVL measures were derived from the HIV-positive males (Models A) and the HIV-positive and HIV-negative males (Models B) of a population-based survey. The full output is given in Tables S4 and S5 of the supplementary materials.

HIV acquisition risk for females	**Geometric mean viral load***			**Percentage detectable viremia†**			**Community transmission index‡**		
	HR	(95% CI)	p-value	HR	(95% CI)	p-value	HR	(95% CI)	p-value
Population-based: HIV-positive males only									
Model A1: Unadjusted HR	1.000	(1.000-1.000)	0.393	1.000	(0.994-1.006)	0.983	0.999	(0.987-1.012)	0.922
Model A2: Adjusted HR without HIV prevalence	1.000	(1.000-1.000)	0.836	1.001	(0.995-1.008)	0.662	1.003	(0.991-1.016)	0.599
Model A3: Adjusted HR with HIV prevalence	1.000	(1.000-1.000)	0.487	1.003	(0.996-1.011)	0.393	1.004	(0.990-1.018)	0.563
Population-based: HIV-positive and HIV-negative males									
Model B1: Unadjusted HR	1.066	(1.021-1.113)	0.004	1.039	(1.016-1.063)	0.001	1.100	(1.033-1.171)	0.003
Model B2: Adjusted HR without HIV prevalence	1.056	(1.006-1.108)	0.027	1.039	(1.013-1.066)	0.003	1.095	(1.025-1.171)	0.007
Model B3: Adjusted HR with HIV prevalence	1.160	(1.058-1.271)	0.001	1.061	(1.020-1.104)	0.004	1.110	(1.015-1.214)	0.022
N	5188			5188			5188		

Exponentiated coefficients. *For a one unit increase in the geometric mean viral load. †For a one percent increase in the prevalence of detectable viremia. ‡ For a one transmission event increase per 100 sexual contacts.

Table 4B: Results of the multivariable analysis (Cox Proportional Hazard model) to examine the relationship between the risk of HIV acquisition for males and the three female community viral load (PVL) measures. The PVL measures were derived from the HIV-positive females (Models A) and the HIV-positive and HIV-negative females (Models B) of a population-based survey. The full output is given in Tables S6 and S7 of the supplementary materials.

HIV acquisition risk for males	**Geometric mean viral load***			**Percentage detectable viremia†**			**Community transmission index‡**		
	HR	(95% CI)	p-value	HR	(95% CI)	p-value	HR	(95% CI)	p-value
Population-based: HIV-positive females only									
Model A1: Unadjusted HR	1.000	(1.000-1.000)	0.176	0.985	(0.965-1.007)	0.174	0.977	(0.920-1.037)	0.439
Model A2: Adjusted HR without HIV prevalence	1.000	(1.000-1.000)	0.989	0.997	(0.972-1.021)	0.782	1.010	(0.943-1.082)	0.773
Model A3: Adjusted HR with HIV prevalence	1.000	(1.000-1.000)	0.985	0.997	(0.971-1.024)	0.809	1.008	(0.933-1.088)	0.845
Population-based: HIV-positive and HIV-negative females									
Model B1: Unadjusted HR	1.057	(1.030-1.084)	<0.001	1.109	(1.052-1.170)	<0.001	1.405	(1.174-1.683)	<0.001
Model B2: Adjusted HR without HIV prevalence	1.053	(1.009-1.099)	0.018	1.077	(1.004-1.155)	0.039	1.270	(1.020-1.581)	0.032
Model B3: Adjusted HR with HIV prevalence	1.081	(1.009-1.158)	0.028	1.106	(1.001-1.221)	0.048	1.452	(1.057-1.997)	0.021
N	3544			3544			3544		

Exponentiated coefficients. *For a one unit increase in the geometric mean viral load. †For a one percent increase in the prevalence of detectable viremia. ‡ For a one transmission event increase per 100 sexual contacts.