

Higher HIV-1 Incidence and Genetic Complexity Along Main Roads in Rakai District, Uganda

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Objective: To determine the association between the incidence of HIV-1 infection and the genetic complexity of HIV-1 strains in 2 geographic strata within Rakai District, Uganda.

Methods: Study volunteers with recent HIV-1 infections during the period 1997 through 2003 were recruited from 10 communities that were geographically stratified as a main road trading center (n = 5) or a secondary road trading village (n = 5). Cryopreserved plasma was available from 384 volunteers and was the source of viral RNA for genotyping by the multiregion hybridization assay. Hazard ratios (HRs) for a single HIV subtype, a recombinant form, or dual infection for gender and geographic strata were obtained using Cox proportional hazards analysis.

Results: The HIV-1 incidence rate during the period 1999 through 2002 was 1.3 per 100 person-years (PYs) in the trading centers and 1.1 per 100 PYs in the trading villages. The HR for infection with an HIV-1 recombinant strain in trading centers relative to trading villages was 2.3 (95% confidence interval [CI]: 1.0 to 6.7). Among those who changed residence between village strata, the HR for a recombinant HIV-1 infection was 8.1 (95% CI: 0.4 to 47.7).

Conclusions: HIV-1 incidence and genetic complexity are associated with geographic strata and population mobility in Rakai District and are important variables to be considered in planning and recruitment for vaccine trials.

Key Words: HIV-1, molecular epidemiology, Uganda

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The molecular epidemiology of HIV-1 reveals a complex evolving pandemic, composed of 9 genetic subtypes, 20 or more circulating recombinant forms (CRFs), and myriad unique recombinant forms (URFs).¹ Viral variation must be accurately documented to assess its impact on treatment prevention,² and the social and geographic dimensions of the epidemic are particularly important for the planning of HIV-1 vaccine trials.³

In many potential vaccine trial sites in Africa, intersubtype recombination is an important factor influencing the complexity of HIV-1 strains. The origin and genesis of recombinant strains are being actively investigated to gain insight into associated population demographic variables. In urban and rural areas of the Mbeya region in Tanzania, an association between HIV-1 prevalence and viral diversity has recently been reported.⁴ Individuals living in high-prevalence urban areas were more likely to be infected with a recombinant strain and to be dually infected compared with individuals from a lower prevalence rural village. The urban community had greater access to the TransAfrican highway, where residents often engage in social interactions, with increased opportunity for casual sexual encounters between travelers and local inhabitants.^{5,6} In the Rakai District of Uganda, previous studies have also reported differences in HIV-1 prevalence and risk factors associated with the sexual transmission of HIV-1 within geographic strata of differing accessibility to main roads.^{7,8}

A previous study in Rakai documented that subtype D accounts for more than 50% of infections and subtype A for <20%, whereas almost 30% of HIV-1 infections in Rakai are intersubtype recombinant strains.⁹ We hypothesized that communities along main roads in Rakai District, compared with their more rural and less accessible counterparts, would provide an environment for increased social and sexual

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interaction, higher HIV-1 incidence, increased importation and transmission of novel HIV-1 strains, and increased risk for multiple exposures to HIV-1, leading to dual infection and generation of intersubtype recombinant strains. Here, we report an analysis of HIV-1 incidence and demographic variables in 10 communities in the Rakai District of Uganda and the genotype of HIV-1 strains in 329 recently infected individuals from these communities.

METHODS

Study Population

The population in Rakai District has been extensively studied for HIV incidence and risk factors since 1988 through a series of community cohort studies. These cohort studies are annual surveys of selected trading center communities and rural village communities representing a cross section of the Rakai population. An average of 19,228 volunteers participated annually. Individuals who had a negative HIV test result followed by a positive HIV test result within any of 3 successive cohort studies were recruited to the Molecular Epidemiology (MER) study within 1 month of their first positive HIV test result. The MER study recruited 80 of these incident cases from the 1997 through 1998 study interval, 188 from 1999 through 2002, and 140 from 2002 through 2003. Among this total population of recent seroconverters (n = 408), 384 had a cryopreserved plasma sample from entry into the MER study available for analysis.

MER study participants were offered posttest counseling by the cohort staff and were referred to MER study staff for study-specific informed consent, which was obtained in Lugandan. This research project and the consent documents were approved by the Uganda Viral Research Institute Institutional Review Board (IRB), the IRBs of Columbia University and Johns Hopkins School of Public Health, and the US Army Surgeon General’s Human Subjects Research Review Board.

MER study participants resided in 1 of 10 different communities within the Rakai District as previously described by Wawer et al⁷ (Fig. 1). The main road trading centers (Katana, Kalisizo, Kyotera, Kasasa-Sanje, and Kakuuto) included shops, bars, and hotels, all serving domestic and international traffic. In contrast, the villages (Lwamaggwa, Kabira, Buyamba, Lwanda, and Kibale-Rakai) are 2 to 10 km from main roads and accessible only via gravel or dirt roads.

For determination of HIV-1 serostatus within the cohort studies, sera were tested with dual enzyme-linked immunosorbent assay (ELISA) tests (Vironostika, HIV Uni-Form II Test, Durham, NC; Abbott Wellcozyme HIV Recombinant Test [Abbott Laboratories, North Chicago, IL]) and were confirmed by HIV-1 Western blot (Calypte/Cambridge Biotech HIV-1, Berkeley, CA).

HIV-1 Incidence

HIV-1 incidence rates were calculated only for the cohort study from 1999 through 2002, because the remaining 2 studies are ongoing or part of separate reports. This was part of the MER study; it was called the Community HIV Epidemiology Research (CHER) study and was similarly

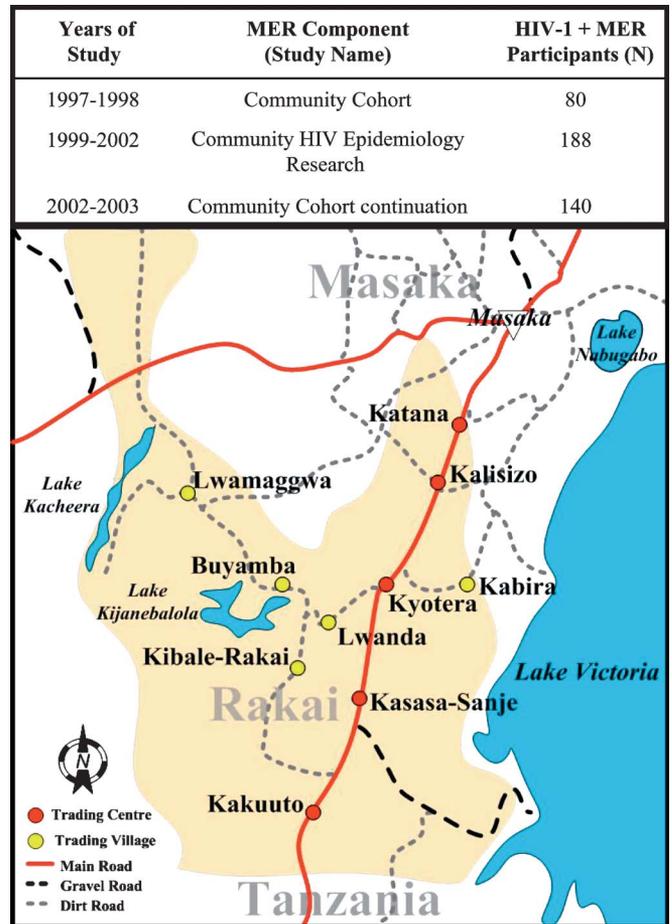


FIGURE 1. Population geography and community stratification for the MER study. Individuals with incident HIV-1 infections from these studies in Rakai, spanning 1997 through 2003, volunteered to participate in the MER study as indicated. Volunteers lived in 1 of 10 communities, which were stratified as a main road trading center (red dots) or as a trading village (yellow dots).

conducted, with all participants providing informed consent under protocols approved by local and US IRBs as described previously. In this study, 188 incident cases were identified during 3 annual surveys in the 10 study communities. The total number of person-years (PYs) was calculated separately for 2 groups: based on interval of years between the first and last visits for HIV-1–negative individuals and based on seroconverters contributing person-time from their last negative HIV test result to their first positive HIV test result. We defined this as the midpoint between the 2 dates, and seroconverters were presumed to contribute person-time to the cohort follow-up from their first negative HIV test result to the midpoint date of their seroconversion interval. The incidence rates presented are unadjusted, because the calendar period was too short to determine any meaningful trends (3 years), with numerous individuals participating in only the first 2 years or the last 2 years. Incidence rates were calculated as the number of seroconversions per 100 PYs of follow-up, along with the 95% confidence interval (CI).

Multiregion Hybridization Assay version 2

Viral RNA was extracted from 200 μ L of plasma using the MagNA Pure Total Nucleic Acid robotic extraction procedure (Roche Diagnostics Corporation, Indianapolis, IN). As negative controls, phosphate-buffered saline (PBS) and HIV-1-seronegative samples were included in each extraction procedure and in the multiregion hybridization assay (MHAacd) analysis. The MHAacd was performed as described by Arroyo et al.^{4,10,11}

Statistical Analysis

The 2 geographic strata were compared for population demographic characteristics and HIV subtype proportions using the χ^2 test or Fisher exact test. Only significant *P* values <0.05 are shown. Adjusted odds ratios (AORs) were calculated, adjusting for those variables that were statistically significantly predictive of HIV risk on bivariate analysis, gender, age, strata, and seroconversion time period so as to determine any associations with HIV-1 subtype. Cox proportional hazards regression analysis was used to calculate HIV-1 subtype incidence hazard ratios (HRs) along with their 95% CIs. All statistical analyses were performed using JMP 5.1 software (SAS Institute, Cary, NC).

RESULTS

Characteristics of Molecular Epidemiology Study Participants

A total of 408 individuals with incident HIV infection observed within ongoing community cohort studies from 10 communities in Rakai District were enrolled into the MER study over the period 1997 through 2003 (Fig. 1). Two hundred thirty-five (58%) resided in 1 of 5 trading centers along the TransAfrican highway leading to Tanzania, whereas 173 (42%) came from trading villages along secondary roads. Of all the MER study volunteers, 246 (60%) were women (56% lived in trading centers and 44% in trading villages) and 162 (40%) were men (60% lived in trading centers and 40% in trading villages). Each community enrolled male and female participants in similar numbers, except Kakuuto, which enrolled fewer female participants (*P* = 0.05). There was no significant difference in gender ratio between strata (trading center vs. trading village). The age of enrollees at first enrollment over the 3-year period ranged from 14 to 90 years, with a mean age of 28.5 years and a median age of 26 years; the interquartile range was 21 to 34 years. HIV-positive volunteers were significantly (*P* < 0.05) older than HIV-negative volunteers by 2.5 years (31.1 vs. 28.6 years, respectively). Men and women had a similar age distribution (mean age of 28.5 years, SD = 8.0 for women, mean age of 31.44 years, SD = 6.9 for men) in the 2 strata.

HIV-1 Subtype Distribution

Plasma samples collected within 1 month of the first HIV-1-seropositive sample were available from 384 (94%) of 408 participants, and of these, 329 (86%) were successfully genotyped using the MHAacd (Table 1). Overall, the cohort had 52 (16%) subtype A, 189 (57%) subtype D, and 87 (26%)

recombinant HIV-1 strains. The only subtype C strain detected was from Kyotera main road trading center, representing <1% of the total subtype distribution. The distribution among the recombinant strains was 3 (3%) AC, 68 (78%) AD, 14 (16%) CD, and 2 (2%) ACD. Among all samples genotyped, 273 (83%) were subtype D or a subtype-D-containing recombinant strain. The proportions of subtype D differ by 8% between the 2 geographic strata (subtype D, 54% vs. 62%). Kakuuto had more subtype D (71%) and Kibale-Rakai had more subtype A (29%) than other communities. Main road trading centers had more recombinant strains than trading villages (32% vs. 20%; *P* < 0.05). Recombinants constituted 32% to 35% of strains in 4 trading centers, but Kakuuto was the exception, with only 14% recombinants. In trading villages, the proportion of recombinant strains averaged 20%, ranging from 15% to 23%.

Dual infections, ascertained by 2 or more subtype-specific probes hybridizing in a given genome region, were found in 23 (7%) of participants (Table 1) at rates of 7.5% in trading centers and 6.3% in trading villages. Most dual infection cases detected occurred between AD (87%) subtypes, whereas only a few occurred between CD (9%) and ACD (4%) subtypes. These results showed an increase in the number of HIV recombinant strains and dual infections in trading centers, which was also associated with a decrease in the number of subtype D strains. These differences represent an increase in the genetic complexity of HIV strains circulating in this stratum. To determine the significance of these differences between the 2 strata, we categorized HIV-1 genotypes in subsequent analyses as subtype D versus non-subtype D, pure subtype versus recombinant, or dual versus single infection, respectively.

Multivariate Regression Analysis

Multivariate regression analysis was performed to calculate the odds ratio for a particular HIV-1 subtype among those MER study participants who were successfully genotyped (*n* = 329). The unadjusted odds ratios and AORs for recombinant, nonsubtype D, and dual infections, by gender and geographic stratum, are shown in Table 2. The analysis confirms increased odds ratios for recombinant HIV-1 strain infection among men and women in trading centers compared with trading villages (AOR among women = 1.7, 95% CI: 0.9 to 3.5; AOR among men = 2.0, 95% CI: 0.8 to 4.8). The risk of non-subtype D infections was increased among men in trading centers (AOR = 1.7, 95% CI: 0.8 to 3.6), whereas among women in trading centers, there was also an increase in the risk of a dual HIV-1 infection (AOR = 1.8, 95% CI: 0.5 to 7.8). These results are noteworthy, but the lack of statistical significance might be accounted for by the small number of samples analyzed.

HIV-1 Incidence

The CHER study conducted between 1999 and 2002 identified 188 incident cases (Fig. 1). HIV-1 incidence rates stratified by gender and geography are shown in Table 3. The overall incidence was 1.3 per 100 PYs. Women had a higher HIV-1 incidence rate than men (1.3 vs. 1.2 per 100 PYs). The HIV-1 incidence rate was higher in the main road trading

TABLE 1. HIV-1 Subtype Distribution in MER Study Participants

Stratum	Community	No. Samples Genotyped	HIV-1 Subtype Distribution (%)				Dual Infection (%)
			A	C	D	Recombinants	
Secondary road trading villages	Lwamaggwa	13	23	0	62	15	0
	Buyamba	39	10	0	67	23	5.1
	Lwanda	25	20	0	64	16	4.0
	Kabira	35	14	0	66	20	11.4
	Kibale-Rakai	31	29	0	52	19	6.5
	Subtotal*	143	18	0	62	20	6.3
Main road trading centers	Katana	46	15	0	50	35	8.7
	Kalisizo	35	14	0	51	34	11.4
	Kyotera	31	19	3	42	35	3.2
	Kasasa-Sanje	53	9	0	58	32	7.5
	Kakuuto	21	14	0	71	14	4.8
	Subtotal*	186	14	1	54	32	7.5
Both strata	Total†	329	16	0	57	26	7.0

*Total and average values representative of all the communities within each respective stratum.

†Total and average values representative of all the communities within each respective stratum.

centers compared with trading villages in men and women (men: 1.3 vs. 1.2 per 100 PYs; women: 1.3 vs. 1.1 per 100 PYs). The trading center incidence was also higher than that of trading villages overall (1.3 vs. 1.1 per 100 PYs). The HIV-1 incidence was significantly higher among individuals who moved between strata (6.1 per 100 PYs) versus those who remained in trading villages (1.1 per 100 PYs; $P < 0.01$) or trading centers (1.3 per 100 PY; $P < 0.01$) at any time during the CHER study. This migration-associated increased incidence was higher among women (9.2 per 100 PYs; $P < 0.01$) than men (1.8 per 100 PYs).

Hazard Ratios of HIV-1 Infection by Genotype and Geographic Stratum

The HRs for HIV-infection with a particular HIV-1 strain (subtype D, pure subtype [A, C, or D], non-D subtype, and

recombinant) were calculated among those individuals who seroconverted ($n = 188$) during 1999 through 2002 (Table 4). The risk for subtype D infection varied little by gender or community type. Considering pure subtype infections (A, C, or D strains) collectively, however, women who moved between strata had a higher risk (HR = 5.7, 95% CI: 1.4 to 16.2). Similarly, grouping the non-D strains together (A, C, and recombinant) showed elevated risk among women in trading centers (HR = 2.3, 95% CI: 1.0 to 5.9), and especially among those who were mobile between strata (HR = 20.6, 95% CI: 4.4 to 74.7).

The risk of a recombinant infection was also elevated in women living in trading centers (HR = 5.3, 95% CI: 1.5 to 34.2) or in those who moved between strata (HR = 22.9, 95% CI: 1.1 to 241.0) compared with women who lived in rural trading villages. Of interest, this disproportionate risk for

TABLE 2. Odds Ratios for Recombinants, Non-Subtype D Strains, and Dual Infections

Comparison Groups	Women		Men	
	OR (95% CI)	AOR* (95% CI)	OR (95% CI)	AOR (95% CI)
Recombinant vs. nonrecombinant strains				
Trading villages†	1.0	1.0	1.0	1.0
Trading centers‡	1.8 (1.0 to 3.6)	1.7 (0.9 to 3.5)	2.1 (0.9 to 5.0)	2.0 (0.8 to 4.8)
Non-subtype D vs. subtype D strains				
Trading villages	1.0	1.0	1.0	1.0
Trading centers	1.3 (0.7 to 2.2)	1.2 (0.7 to 2.2)	1.7 (0.9 to 3.6)	1.7 (0.8 to 3.6)
Dual infection vs. single infection				
Trading villages	1.0	1.0	1.0	1.0
Trading centers	1.5 (0.4 to 5.8)	1.8 (0.5 to 7.8)	1.1 (0.3 to 3.7)	0.8 (0.2 to 3.1)

Statistically significant OR values ($P < 0.05$) are shown in bold.

*AOR by age and seroconversion time period.

†Secondary road trading village (Lwamaggwa, Buyamba, Lwanda, Kabira, and Kibale-Rakai).

‡Main road trading center (Katana, Kalisizo, Kyotera, Kasasa-Sanje, and Kakuuto).

OR indicates odds ratio.

TABLE 3. HIV-1 Incidence Rates in CHER Study Participants

Group	Men		Women	
	Incident Cases/No. PYs	Incident Rate per 100 PYs (95% CI)*	Incident Cases/No. PYs	Incident Rate per 100 PYs (95% CI)*
Trading village†	36/3132	1.2 (0.8 to 1.5)	43/3872	1.1 (0.8 to 1.4)
Trading center‡	43/3366	1.3 (0.9 to 1.7)	58/4313	1.3 (1.0 to 1.7)
Moved between stratum§	1/55	1.8 (0.0 to 5.4)	7/77	9.2 (2.7 to 15.6)
Overall	80/6553	1.2 (1.0 to 1.5)	108/8262	1.3 (1.1 to 1.6)

*HIV-1 incidence rate per 100 PYs at 95% CI in the CHER study.

†Trading village (Lwamaggwa, Buyamba, Lwanda, Kabira, and Kibale-Rakai).

‡Trading center (Katana, Kalisizo, Kyotera, Kasasa-Sanje, and Kakuuto).

§CHER study participants who changed place of residence between strata at any time during the study.

||All category groups combined.

acquisition of certain HIV-1 strains, namely, those other than the majority subtype D strain, was not observed among men.

DISCUSSION

Through the development and application of high-throughput genotyping assays like the MHAacD, the social and geographic dimensions of HIV-1 epidemics can be described with new clarity and the molecular epidemiology of HIV-1 in cohorts with different levels of risk can be assessed. This study adds to a growing body of evidence that the risk for HIV-1 infection, and the social milieu in which infection takes place, can have an impact not only on HIV-1 incidence but on the genetic complexity of HIV-1 strains in the population. In a larger study conducted previously throughout Rakai District, the association of higher HIV-1 prevalence with proximity to paved roads was demonstrated, suggesting that HIV-1

transmission tends to follow lines of transportation, communication, and social interaction.^{7,8} A recent study by Arroyo et al⁴ in the Mbeya region of Tanzania reiterated this finding and added another dimension, associating higher prevalence with a higher genetic complexity of strains. Other studies have also found this relation when comparing high- and low-risk cohorts.^{5,12} This study confirms an association between increased exposure to HIV-1 strains and the development of HIV-1 genetic complexity.

One correlate of these observations could be a decline in the genetic complexity of HIV-1 when prevalence rates fall. Yirell et al,¹³ studying another region of Uganda, recently reported a parallel decline in the prevalence and fraction of recombinant strains over 10 years. The low HIV-1 incidence reported in the CHER study was in accordance with previous reports in southwest Uganda.^{14,15} The challenge of depicting differences in HIV-1 incidence in a low-risk population might

TABLE 4. Unadjusted HRs for HIV-1 Infection by Genotype and Geographic Stratum

Variable	Men		Women	
	No. Incident Cases	HR (95% CI)*	No. Incident Cases	HR (95% CI)*
D subtype				
Trading villages†	10	1.0	21	1.0
Trading centers‡	12	1.1 (0.5 to 2.7)	20	0.9 (0.5 to 1.6)
Moved between strata§	0	0.0	1	2.3 (0.1 to 11.1)
Pure subtype (A, C, D)				
Trading villages	13	1.0	26	1.0
Trading centers	17	1.1 (0.6 to 2.6)	26	0.9 (0.5 to 1.6)
Moved between strata	0	0.0	3	5.7 (1.4 to 16.2)
Non-D (A, C, recombinant)				
Trading villages	7	1.0	7	1.0
Trading centers	9	1.2 (0.4 to 3.3)	18	2.3 (1.0 to 5.9)
Moved between strata	0	0.0	3	20.6 (4.4 to 74.7)
Recombinant				
Trading villages	4	1.0	2	1.0
Trading centers	4	0.92 (0.2 to 3.9)	12	5.33 (1.5 to 34.2)
Moved between strata	0	0.0	1	22.9 (1.1 to 241.0)

Statistically significant HRs are shown in bold ($P < 0.05$).

†Trading village (Lwamaggwa, Buyamba, Lwanda, Kabira, and Kibale-Rakai).

‡Trading center (Katana, Kalisizo, Kyotera, Kasasa-Sanje, and Kakuuto).

§MER study participants who changed residence between strata in CHER study.

have accounted for the lack of statistical significance observed. Despite this fact, we were able to observe subtle differences in HIV-1 incidence in different population strata, and these were associated with differing HIV-1 subtype complexity.

With respect to planning for vaccine trials, the results reported here as well as others reported previously confirm that there is considerable heterogeneity with respect to incidence and subtype complexity within potential source populations for vaccine trials. Finding the optimal balance between high HIV-1 incidence, which simplifies trials, and the complexity of the HIV-1 strains that challenge volunteers, which may complicate the interpretation of trial results, can be facilitated by studies that reveal the complex interactions between gender, social setting, and mobility in potential trial sites.

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