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COMPARISON OF PLASMA VIRAL LOAD AND OTHER PREDICTORS OF TRANSMISSION FROM MEN TO WOMEN AND WOMEN TO MEN IN DISCORDANT COUPLES FROM RWANDA (CLADE A HIV) AND ZAMBIA (CLADE C HIV)

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By

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Bachelor of Arts
St. Olaf College
2007

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ABSTRACT

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OTHER PREDICTORS OF TRANSMISSION
FROM MEN TO WOMEN AND WOMEN TO MEN IN DISCORDANT COUPLES
FROM RWANDA (CLADE A HIV) AND ZAMBIA (CLADE C HIV)

By Chad Stegeman

A paper by Fideli et al. analyzed factors of HIV transmission among couples in the RZHRG cohort from 1995 to 2000 and found that viral load contributed a significantly larger risk to HIV transmission in woman-to-man (FTM) transmission couples than in man-to-woman (MTF) transmission couples. This paper aims to understand how gender differences in factors of HIV transmission have changed with an additional 12 years of follow-up data. The results showed that controlling for couples' age, genital ulcers, and unprotected coital acts, the viral load estimates for FTM and MTF couples were not statistically different at the 0.05 level with a p-value of 0.309. Further analysis of data that included couples from Ndola, Zambia and Kigali, Rwanda revealed genital ulcers increase the risk of HIV transmission by a factor of 3.14 (2.38, 4.15) for FTM couples, but by a factor of only 2.11 (1.62, 2.74) for MTF couples. The analysis also showed a couple being Zambian compared to Rwandan increased the risk of transmission more for MTF couples than FTM couples. Understanding these gender differences could be an important step in understanding the HIV-1 epidemic in sub-Saharan Africa.

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LITERATURE REVIEW

Significance of HIV

Globally, there were an estimated 33.3 million people living with Human Immunodeficiency Virus (HIV) at the end of 2009 with an estimated two-thirds of those residing in sub-Saharan Africa. A majority of infections in this region of the world is caused by heterosexual transmission (1). In Zambia and Rwanda it is estimated that between 55.1% and 92.7% of new heterosexual HIV infections are acquired from a spouse or other cohabitating partner (2). The large concentration of disease in one area poses a huge public health problem. Understanding how and when HIV transmission occurs will be beneficial to those providing public health interventions in the area.

HIV: the Virus

HIV is a retrovirus that infects and destroys CD4 T Cells that initiate the immune response toward a new infection. While the body will respond and attempt to destroy infected CD4 T Cells, this immune response will provide new targets for the virus thus driving viral replication. While there is no cure, the use of antiretroviral therapy (ART) will block virus replication allowing the immune system to return to normal function (3).

There are two types of the virus, HIV-1 and HIV-2. These two types along with simian immunodeficiency virus (SIV) make up the subgenus 'primate lentiviruses.' Both types of HIV likely originated from a zoonotic transmission of SIV in primates. HIV-1 seems to have come from SIV in chimpanzees while HIV-2 is more closely associated with SIV of sooty mangabeys (4). HIV-1 has been found to be significantly more

infectious than HIV-2 (5). As a result, HIV-2 is mostly confined to Western Africa and a majority of infections in the world are HIV-1 (4,5).

In replication of HIV, an enzyme called Reverse Transcriptase transcribes viral RNA to cDNA. This process is highly prone to error resulting in large genetic variation within the HIV-1 type and even within individuals (6). HIV-1 strains fall into 3 groups of viruses M (major), O (outlier), and N (novel). The HIV pandemic today is caused by the M group of HIV-1 viruses that have 9 subtypes and 4 combinations of those subtypes (6–9). Genetic variation within a subtype can reach 15%-20%. Between subtypes genetic variation is usually 25% to 35% (9).

HIV-1 subtype B, also referred to as clade B, is the predominant subtype in North America, Australia, and Europe. For this reason, it is the most studied subtype despite only 10.2% of all HIV cases globally being subtype B (6,9). Subtypes A and C are currently the most prevalent. Subtype C is found predominantly in Eastern and Southern Africa and in India. 49.9% of all HIV infections are subtype C. Subtype A is mainly in Eastern and Central Africa, Central Asia, and Eastern Europe. Only 12.3% of HIV infections are of Subtype A (9).

Subtype Differences

Differences in transmission rates among different HIV-1 subtypes have been difficult to define. "Most studies are characterized by small numbers, short follow-up, use of controls derived from different cohorts, and broad comparisons between one subtype and other subtypes grouped together (for example B versus 'non-B')" (7). While an early paper found no differences in rate of CD4 cell decline, clinical progression, or

plasma HIV-1 RNA levels (viral loads) between infected individuals with subtypes A, B, C, and D, the study was small with under 120 individuals total between the four subtypes (6).

One study found that pregnant women infected with subtype C were more likely to shed HIV-1 infected vaginal cells than those infected with subtypes A or D (10). This could imply a higher rate of sexual transmission for subtype C (9). Understanding this HIV subtype diversity and its consequences on transmission will lead to better HIV prevention strategies.

HIV Transmission

Understanding heterosexual HIV transmission is important because it is the most common mode of transmission worldwide (1). Specifically an estimated 55.1% to 92.7% of heterosexually transmitted HIV is between spouses or cohabitating partners (2). While transmission rates for different subtypes have not been well characterized, other factors of HIV transmission such as viral load, gender, age, and STIs have been investigated more thoroughly.

Viral load may be one of the most widely researched modes of HIV transmission. It has been shown that higher viral loads increase HIV transmission between heterosexual couples (11–13). Current anti-retroviral therapies (ARTs) will decrease viral loads and thus decrease transmission. Because ARTs often decrease viral load to below 400 RNA copies/mL and so few transmissions have been recorded when the donor has such low viral loads, there has been difficulty in characterizing transmission rates while couples

are on ARTs (14). This research highlights the importance of controlling for viral loads in our study.

Because the mechanism of heterosexual transmission involves unprotected coital acts, it is important to control for this in studies as well. While collecting this information requires the couples to report honestly and transmission rates per coital act are difficult to estimate, it is still important to control for this (15). For this reason, age is also controlled for as younger people are often more sexually active than older couples(12). Similarly, genital ulcerative disease is an important mechanism in HIV transmission between heterosexual couples. However, publication bias of statistically significant results makes this factor hard to quantitatively describe(16).

While most European and North American studies have found male-to-female transmission slightly more efficient than female-to-male transmission, one study in Africa found female-to-male transmission per coital act to be higher (15). Similarly it was found that with a higher plasma viral loads, female-to-male transmission had a much higher risk of transmission while male-to-female transmission was only weakly associated with seroconversion (17).

RZHRG

An estimated 70% of HIV infections in Zambia are contracted from spouses. It is likely that half to two-thirds of these infections avoidable through joint testing and counseling (2). The Rwanda Zambia HIV Research Group (RZHRG) began by providing HIV testing services as Project San Francisco (PSF) in Rwanda in 1986 called Couples

Voluntary Counseling and Testing (CVCT). In 1994, they created the Zambia-Emory HIV Research Project (ZEHRP), which established CVCT services in Lusaka, Zambia.

ZEHRP has established permanent testing locations and medical facilities in Lusaka and Ndola and PSF has established facilities in Kigali. The projects provide training for Zambian and Rwandan government nurses who perform HIV testing and counseling in clinics throughout the area. Following their training, these nurses work part-time on the weekends for ZEHRP and PSF. The organization advertises for couples to get HIV tested in certain clinics on the weekends, and the nurses that are trained and paid by ZEHRP test the couples, counsel them, and record data.

Previous Research with RZHRG Cohort

In 2001 a paper by Fideli et al. used the same cohort to investigate the factors of HIV transmission. Understanding the cohort and the methods used to address a similar question will provide useful insight as I begin investigating my question.

The RZHRG cohort is a prospective cohort in Lusaka, Zambia, Ndola, Zambia, and Kigali, Rwanda. This paper used only seroconversions that took place in Lusaka, Zambia where more than 90% of HIV infections were subtype C. With subtype A being more prevalent in Rwanda, these seroconversions will allow a comparison between subtype A and C for this research.

At the time of this study, the cohort had 1022 couples where one partner was positive and one partner was negative ("discordant couple"). Couples tested in Couples Voluntary Counseling and Testing (CVCT) were referred to enroll in the Heterosexual Transmission (HT) study. Eligible couples were discordant for HIV-1, living together for

more than 6 months, and women were younger than 48 years and men were younger than 65 years of age. To continue to be enrolled in the study they could not be on Antiretroviral Therapy (ART) or pregnant.

Couples in the HT study were monitored every 3 months. At each 3-month visit, there was documentation of sexual contact both with and without a condom, an interim medical history and physical exam to check for STIs, and repeat HIV serology for the negative partner. Seroconverting couples were invited back for confirmatory blood testing and counseling. To be sure that HIV was acquired from the partner, epidemiological linkages were examined by sequence analysis of HIV in both partners.

The Fideli et al. paper used a nested case-control design and had 66 male and 43 female transmitters and 114 male and 94 female non-transmitters. Viral loads, viral culture data, and/or CD4+ cell levels were recorded for each couple. Using the Cox regression model, it was found that higher viral loads (>100,000 and 10,000-100,000 RNA copies per mL of plasma) were significantly associated with FTM transmission with risk ratios (RRs) of 7.6 and 4.1, respectively. Meanwhile, MTF transmission was only loosely associated at these higher viral load levels with RRs at 2.1 and 1.2 respectively (17).

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MANUSCRIPT

Comparison of Plasma Viral Load and Other Predictors of Transmission From Men to Women and Women to Men in Discordant Couples From Rwanda (Clade A HIV) and Zambia (Clade C HIV)

Chad Stegeman

Susan Allen MD, MPH

Abstract

A paper by Fideli et al. analyzed factors of HIV transmission among couples in the RZHRG cohort from 1995 to 2000 and found that RNA plasma viral load contributed a significantly larger risk to HIV transmission in woman-to-man (FTM) transmission couples than in man-to-woman (MTF) transmission couples. This paper aims to understand how gender differences in factors of HIV transmission have changed with an additional 12 years of follow-up data. The results showed that controlling for couples' age, genital ulcers, and unprotected coital acts, the viral load estimates for FTM and MTF couples were not statistically different at the 0.05 level with a p-value of 0.309. Further analysis of data that included couples from Ndola, Zambia and Kigali, Rwanda revealed genital ulcers in both transmitter and seroconvertor increase the risk of HIV transmission by a factor of 3.14 (2.38, 4.15) for FTM couples, but by a factor of only 2.11 (1.62, 2.74) for MTF couples. The analysis also showed a couple being Zambian compared to Rwandan increased the risk of transmission more for MTF couples than FTM couples. Understanding these gender differences could be an important step in understanding the HIV-1 epidemic in sub-Saharan Africa.

Introduction

Today there are an estimated 34 million people living with Human Immunodeficiency Virus (HIV) with an estimated two-thirds of those residing in sub-Saharan Africa. A majority of infections in this region of the world are caused by heterosexual transmission (1). In Zambia and Rwanda it is estimated that between 55.1% and 92.7% of new heterosexual HIV infections are acquired from a spouse or other cohabitating partner (2). This large concentration of disease in one area poses a significant public health problem. Understanding HIV transmission and how it differs by gender is vital to those providing public health interventions in the area.

Viral load may be one of the most widely researched modifiers (or determinants) of HIV transmission. It has been shown that higher viral loads increase HIV transmission between heterosexual couples (3–5). Current anti-retroviral therapies (ARTs) decrease viral loads and thus decrease transmission. Because ARTs often decrease plasma viral load to below 400 RNA copies/mL and so few transmissions have been recorded when the donor has such low viral loads, there has been difficulty in characterizing transmission rates while couples are on ARTs (6).

Collecting unprotected coital act and genital ulcerative disease information requires study participants to honestly report their data. Because these factors are important modifiers of heterosexual transmission, it is still important to try to obtain the data and control for these factors. Due to inconsistency in reporting, transmission rates per coital act are difficult to estimate (7). However, one study in Africa found female-to-male (FTM) transmission per coital act to be higher than male-to-female (MTF)

transmission per coital act (7). Genital ulcerative disease can be easier to detect, yet publication bias of statistically significant results has made this factor hard to quantitatively describe (8). Age has been shown to be another factor in transmission of HIV possibly in part because younger people are generally more sexually active than older couples (4).

The HIV pandemic today is caused by the M group of HIV-1 viruses that have 9 clades and 4 combinations of those clades (9–12). Genetic variation within a clade can reach 15%-20%. Between clades genetic variation is usually 25% to 35% (12). HIV-1 clade B, also referred to as subtype B, is the predominant clade in North America, Australia, and Europe. For this reason, it is the most studied clade despite only 10.2% of all HIV cases globally being clade B (9,12). Clades A and C are currently the most prevalent. Clade C is found predominantly in Eastern and Southern Africa and in India. 49.9% of all HIV infections are clade C. Clade A is mainly in Eastern and Central Africa, Central Asia, and Eastern Europe. Only 12.3% of HIV infections are of clade A (12).

An estimated 70% of HIV infections in Zambia are contracted within marriage. It is likely that half to two-thirds of these infections are avoidable through joint testing and counseling (2). The Rwanda Zambia HIV Research Group (RZHRG) began by providing HIV testing services as Project San Francisco (PSF) in Rwanda in 1986 called Couples Voluntary Counseling and Testing (CVCT). In 1994, they created the Zambia-Emory HIV Research Project (ZEHRP), which established CVCT services in Lusaka, Zambia.

A paper by Fideli et al. analyzed factors of HIV transmission among couples in this cohort in 2001 and found that viral load contributed a significantly larger risk to HIV

transmission in FTM couples than in MTF couples in Zambia. Using the Cox regression model, it was found that higher viral loads (>100,000 and 10,000-100,000 RNA copies per mL of plasma) were significantly associated with FTM transmission with risk ratios (RRs) of 7.6 and 4.1, respectively. Meanwhile, MTF transmission was only loosely associated at these higher viral load levels with RRs at 2.1 and 1.2 respectively (13).

Methods

The RZHRG Heterosexual Transmission (HT) cohort is a prospective cohort in Lusaka, Zambia; Ndola, Zambia; and Kigali, Rwanda. Cohabitating couples tested in Couples Voluntary Counseling and Testing (CVCT) with one HIV positive individual and one HIV negative individual (discordant couples) were enrolled in the HT study (14). Eligible couples were discordant for HIV-1, living together for more than 6 months, and women were younger than 48 years and men were younger than 65 years of age. This age restriction limited the study to enrollees who would be most sexually active. To be included in the analyses presented here, they could not be on Antiretroviral Therapy (ART). 5,056 discordant couples were enrolled in the study between February 1995 and August 2011.

Emory's Institutional Review Board (IRB) and the Zambian Ethics Committee approved the HT study in which this analysis took place. The RZHRG offices approved of this analysis and considered it to be covered under existing protocols. Informed consent was obtained at enrollment for all study participants at site locations in Zambia and Rwanda.

Couples in the HT study were monitored every 3 months. At each 3-month visit, there was documentation of sexual contact both with and without a condom, an interim medical history and physical exam at baseline and as clinically indicated to check for STIs, and repeat HIV serology for the negative partner. Seroconverting couples were invited back for confirmatory blood testing and counseling.

To be sure that HIV was acquired from the partner, genetic epidemiological linkages were examined by sequence analysis of HIV in both partners after a seroconversion occurrence. In this analysis, linked couples were determined to be transmitting events. Couples that dropped out of the study, began ART treatments before seroconverting, or were determined to be unlinked were censored at time of qualifying event. Couples where linkage could not be determined were dropped from analysis because their factors of transmission could not be attributed to factors of a transmitting couple or a non-transmitting couple. HIV clade information was obtained from the genetic analysis used to confirm epidemiological linkages.

Time independent variables used for this analysis included the country of the site (either Zambia or Rwanda) and the gender of the transmitting partner. The log of the transmitter's viral loads, couple age, ulcer information, and unprotected coital acts were treated as time dependent variables.

The log 10 of the transmitter's viral load was used for a couple for the duration of time that they were enrolled in the study. If multiple viral loads were performed for a couple, the value of the first viral load remained until the point in time that the second viral load was obtained. Donors may have had as many as 13 viral loads done at different

times during the study before their censoring or seroconversion. If two viral loads were done on the same individual from the same time point, an average viral load was used.

The average of the man's age and woman's age was determined at enrollment in the study and increased as their time in the study increased. Average age of the couple was used after determining that either the transmitter's age or the recipient's age was significant in the final model, but together they were not significant. This method utilizes the age information from both couples and was determined to be an appropriate predictor of a couple's sexual habits due to age.

Ulcer information was determined from physical exam forms, 3-month medical forms, and treatment forms collected during the duration of the study. If one partner had a genital ulcer condition, the couple was given an ulcer value of 1 for 90 days prior to the ulcer entry and 30 days after the entry. If both partners had a genital ulcer condition the couple was given an ulcer value of 2 for the same time period. Otherwise, if a couple did not have a genital ulcer condition or did not have information concerning genital ulcers, the couple was given an ulcer vale of 0.

Couples used a calendar to count the number of unprotected coital acts over 3 months of time. If both partners tracked this information and the results differed, an average for the couple was used. At time of transmission, the last available number of unprotected coital acts over 90 days was used. At all other times, the number of recorded acts was used for the 90 days prior to their visit date when this information was recorded.

The data was analyzed using SAS 9.3 to create Extended Cox Proportional Hazards models. Backwards elimination was used starting with a fully parameterized

model along with interaction variables with gender and all other model factors. Due to collinearity, all models with interaction terms were unstable. The final model was stratified, using data that only included women-to-men transmission (FTM), or only included men-to-women transmission (MTF). A Wald statistic with a chi-squared distribution and one degree of freedom was used to compare the model estimates for FTM and MTF couples. The process was repeated using data that only included couples recruited at the Lusaka location, and using only Lusaka couples enrolled before September 1, 2000 to emulate the data used in the Fideli paper.

Results

Table 1 provides summary information for covariates used in the model. There were 5,015 couples from Rwanda and Zambia included in this analysis, 9% of them being a genetically linked seroconversion. In this larger cohort, 86% of Rwanda transmitting couples had clade A HIV, while 98% of Zambian transmitting couples had clade C HIV. In all three data frames, the woman's age is between 25 and 29 years old while the man's age is between 31 and 35 years old. On average the men and women of transmitting couples were between 1 and 3 years younger than non-transmitting individuals of the same gender.

The average number of last reported coital acts is larger in FTM transmitting couples than in non-transmitting couples. In MTF couples, the average is higher for transmitting couples, but the difference is smaller. Across all data frames, this information remains consistent. A larger percentage of transmitting couples reported ever

having unprotected sex with their partner over the duration of the study. With the larger data frames this percentage dropped unilaterally.

The average value of a couple's last ulcer information is larger for transmitting couples than non-transmitting couples in all the data frames. For both FTM and MTF transmitters 76% of couples had a last ulcer value of 0, 21% of couples had a value of 1, and 3% of the couples had an ulcer value of 2. For non-transmitters 94% of couples had a last ulcer value of 0, 6% of couples had a value of 1, and less than 0.5% of couples had an ulcer value of 2.

Table 1b divides the data by partner for those who have ever had an ulcer at baseline or at any other time in the study. In FTM non-transmitting couples, the women are more likely to have had ulcers than the men while in FTM transmitting couples, the percentage of partners ever having reported ulcers is equal between men and women. Similarly in MTF couples, the men (the donor partners) have a larger percentage of ulcers than the women for both non-transmitting couples and transmitting couples. Both transmitting women and transmitting men, and both seroconverting women and seroconverting men were significantly more likely to have an ulcer recorded that their gender-matched counterparts in non-transmitting pairs. There do not seem to be any differences of unprotected coital acts and ulcer information between MTF and FTM transmission.

Table 2 shows the results of the extended cox proportional hazards analysis. The first model shows the analysis using a subset of current data from before September 2000 in Lusaka. Controlling for couple age, genital ulcers, and unprotected coital acts, an

increase of a log10 of the transmitter's viral load for FTM couples increases the risk of transmission 2.35 times with a 95% Confidence Interval of (1.67, 3.31). For MTF couples this increased the risk of transmission was a much lower 1.29 (0.99, 1.67) times. The Wald statistic in Table 2 compares the model estimates for FTM and MTF transmission. At a significance level of 0.05, the analysis confirms that the difference in viral load hazard ratios for FTM and MTF transmission was significantly different ($p=0.006$) for couples recruited in that time frame.

Figure 1 is a box plot that compares viral load differences between men and women transmitters and non-transmitters from this time frame. This figure shows that the difference of last viral load values is larger between women transmitters and women non-transmitters than the difference for transmitting men and non-transmitting men. The table below the plot compares the number of couples that were analyzed in the original publication to the number of couples that were used in the model. While the number of non-transmitters is larger in this analysis and some transmitting couples are missing, the results are the same as in the original publication.

Figure 2 includes only Lusaka couples enrolled in the study after September 1, 2000. This plot shows that shows that men continued to have a higher average viral load than both transmitting and non-transmitting women. Compared to Figure 1, MTF non-transmitting men seem to have a slightly lower average viral load and transmitting men have a slightly higher average viral load.

Figure 3 shows plots using all time data for couples in Lusaka. Table 2 shows an increase in a log viral load of the transmitting partner for FTM couples will increase the

risk of transmission by 1.63 (1.32, 2.00) times and for MTF couples will increase the risk of transmission 1.40 (1.16, 1.70) times. The Wald statistic comparing the model estimates for these factors did not find them to be statistically significant at a 0.05 level (p=0.309).

Figure 4 plots viral loads for couples from Rwanda and shows that Rwandan couples had lower viral loads than couples from Lusaka, Zambia. While the median viral load for transmitting women in Lusaka was 70,000 with a 25%-74% range of (20,000-182,00), the median viral load for transmitting women in Rwanda was 63,000 (10,000-145,000). Similarly non-transmitting women from Lusaka had a higher median viral load of 25,000 (5,400-107,000) compared to non-transmitting women from Rwanda with a viral load of 6,000 (570-24,000). Transmitting and non-transmitting men from Lusaka had higher median viral loads of 161,00 (66,000-389,000) and 82,000 (19,000-214,00) respectively compared to transmitting and non-transmitting median viral loads of 94,000 (18,000-157,000) and 19,000 (3,000-64,000) respectively.

Figure 5 includes data from Lusaka, Ndola, and Kigali. Ndola was not plotted alone due to the low number of viral loads performed on transmitting individuals. Figure 5 affirms the effect of viral load on HIV transmission is equal for women-to-men and men-to-women when all three RZHRG sites were analyzed. The hazard ratio in Table 2 for the log viral load of the transmitting partner in FTM and MTF couples is 1.45 (1.23, 1.71) and 1.47 (1.25, 1.74) respectively.

An interesting finding from this analysis reveals that when all the RZHRG sites for all the time points were analyzed, the effect of ulcer information and country is statistically different for FTM and MTF couples. Table 2 shows that controlling for all

other factors, genital ulcers can increase the risk of HIV transmission by a factor of 3.14 (2.38, 4.15) for FTM couples. However in MTF couples, genital ulcers can increase the risk of HIV transmission by a factor 2.11 (1.62, 2.74). The Wald statistic comparing model estimates confirms that these risk factors are statistically different at the 0.05 level with the p-value = 0.040.

The table also shows that the risk of HIV transmission for FTM couples is increased by a factor of 1.62 (1.15, 2.30) if it is a Zambian couple compared to a Rwandan couple. Although for MTF couples, the risk of HIV transmission for Zambian couples is 2.76 (1.89, 4.04) times higher than Rwandan couples. The Wald statistic comparing model estimates shows that gender differences are statistically significant at the 0.05 level with a p-value of 0.043.

Discussion

This paper utilizes a similar analysis used by Fideli et al on an RZHRG cohort, but the use of certain variables has changed. The original publication utilized time-independent factors based on age, genital ulcers, unprotected coital acts, HIV disease stage, and RNA viral load at the time of transmission or a single time of sample collection for non-transmitting controls. This paper utilizes the time dependent information so that all data from multiple time points for a couple is used. In addition, the original publication explored both categorical and continuous viral loads but reported model results using dummy variables for 3 groups of viral load. This analysis used the log 10 of viral loads as a continuous factor to simplify the number of model results.

Similar to the Fideli publication, this analysis used couples that were known to have genetically linked HIV for the event population, and unlinked couples were censored at time of transmission. The original publication classified 11 unknown linkages as linked couples based on the overall prevalence of linked couples, however these couples were removed from the analysis here. This method of classifying event and censored couples was used to correctly classify the contributing or non-contributing factors to HIV transmission. Despite this difference in analysis, the same conclusions were reached for data from the original publication's time frame. It still holds true that for RZHRG couples enrolled in Lusaka between February 1995 and September 2000 viral load contributed a significantly larger risk to HIV transmission in FTM couples than in MTF couples. However, the cohort has also grown in size considerably.

The original publication contained viral load information for 104 transmitting couples and 207 non-transmitting Lusaka couples. This analysis included 214 transmitting couples and 766 non-transmitting couples from Lusaka enrolled between February 1995 and January 2010. An expanded analysis including couples from Ndola, Zambia and Kigali, Rwanda enrolled between February 1995 and August 2011 contained viral load information for 277 transmitting couples and 1,141 non-transmitting couples. The larger numbers have resulted in a more powerful analysis that found that the effect of viral load on transmission to be the same between MTF couples and FTM couples.

In the model that includes couples recruited from all countries at all time points, two variables were found to be statistically different for FTM and MTF couples. The analysis showed the increased risk of transmission for Zambian compared to Rwandan couples was larger for MTF couples than FTM couples. This difference may be due to

social or cultural differences in each country. It may also be due to the viral clade. A majority of Zambians have clade C HIV, while clade A the predominant clade in Rwanda, and viral loads for all groups were substantially lower in Rwanda compared with Zambia. The country variable may be acting as a proxy for the viral clade C in Zambia and A in Rwanda. Further research is needed to understand the relationship on viral clades and HIV transmission for FTM and MTF transmission.

Additionally, the full analysis showed that, controlling for all other factors, either or both partners having a genital ulcer increased the risk of transmission more for FTM couples than MTF couples. Table 1b shows the stratified summarization of ever having an ulcer between men and women transmitting and non-transmitting couples. Among FTM couples, the percentage of transmitting women ever recording an ulcer was consistently larger than non-transmitting women. Similarly for MTF couples, the percentage of transmitting men ever recording an ulcer was consistently larger than non-transmitting men. Among FTM couples, the percentage of seroconverting men ever recording an ulcer is over twice the percentage of non-seroconverting men. For MTF couples, the percentage of seroconverting women ever recording an ulcer is larger than for non-seroconverting women, but the difference in the percentages is less dramatic.

While it appears that the last ulcer and ever having ulcer information is virtually the same for FTM and MTF couples, this ulcer data in the analysis is treated as time dependent. A couple having an ulcer contributes that information for the 90 days prior to reporting the information and 30 days after reporting the information. It is difficult to infer the cause of different hazard ratios for the ulcer variable for FTM couples and MTF couples from summary statistics alone.

It is possible that men recipients are more susceptible to HIV transmission if an ulcer is involved than women recipients. There may also be bias in the detection of ulcers around the time of transmission that can affect the model estimates. While this difference has not been detected as statistically significant in this dataset before, it is likely that this was due to the study size not being big enough. During the Fideli time frame, the effect of the same ulcer information was larger for FTM couples than MTF couples, but wide confidence intervals made this difference statistically insignificant. Using the largest data frame with the most couples has shown that the effect of an ulcer on HIV transmission is larger for FTM couples than MTF couples.

Although it appears that gender differences in the effect of viral load have disappeared in larger cohorts accumulated over the last decade, viral load is still an important predictor of HIV transmission. With continued ART use out of reach for many couples, research into HIV vaccines has become increasingly important. Differences in HIV transmission risk factors between genders can have a larger impact on the development of vaccines. The effect of country on HIV transmission between genders may be due to cultural differences, but could also likely be due to differences in viral clades found in each country. Moreover, further analysis into the gender differences for the effect of genital ulcerative disease could prove to be an important tool in providing strategic interventions.

Conclusion

While the original study found a gender difference in the effect of viral load on HIV transmission, this study suggests that in a larger cohort of heterosexual couples, controlling for age, genital ulcers, unprotected coital acts, and country of residence the relationship of increasing viral load to increasing likelihood of transmission was the same for both FTM couples and MTF couples. Overall, a log increase in the donor's viral load corresponds to an increased risk by a factor of 1.45 (1.29, 1.62).

In addition, the analysis using the largest cohort found that an ulcer has a larger effect on transmission for FTM couples Hazard Ratio= 3.14 (2.38, 4.15) than for MTF couples HR= 2.11 (1.62, 2.74). The effect of being a Zambian couple compared to a Rwandan couple had a larger effect for MTF couples HR= 2.76 (1.89, 4.04) than for FTM couples HR= 1.62 (1.15, 2.30). Further analysis and research into these differences could be an important step in understanding the HIV-1 epidemic in sub-Saharan Africa.

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We thank the study participants, staff, and interns of the Rwanda Zambia HIV Research Group; the technicians and students at the virology lab at UTH, the Aldrovandi laboratory at UAB, and the Hunter Laboratory at Emory University for completing transmission linkage and HIV viral loads; and the data analysis groups in the Cancer Center at UAB and Allen Office at Emory University for all their help in procuring the necessary data.

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Tables and Figures

Table 1a and 1b.
The tables below show summary statistics for confounding parameters shown an overall model, a female-to-male model, and a male-to-female model. The first data frame includes only couples recruited during the Fideli publication's time frame, February 1995 and September 2000. The second data frame includes only couples recruited at the Lusaka site in Zambia between February 1995 and August 2011. The final data frame includes data from both countries at all time points.

Table 1a.

	Overall		**Female-to-Male**		**Male-to-Female**	
	Non-Transmitting Couples	Transmitting Couples	Non-Transmitting Couples	Transmitting Couples	Non-Transmitting Couples	Transmitting Couples
Lusaka with Fideli Time Points	n=835	n=176	n=409	n=68	n=426	n=108
Donor's Age [α]	31.0 (8.4)	30.0 (8.0)	27.2 (6.9)	25.0 (5.8)	34.6 (8.1)	33.1 (7.7)
Recipient's Age [α]	31.1 (8.8)	28.1 (7.8)	34.4 (9.0)	31.7 (8.4)	27.8 (7.2)	25.8 (6.4)
Average Couple's Age [α]	31.0 (7.4)	29.0 (6.7)	30.8 (7.4)	28.4 (6.5)	31.2 (7.3)	29.5 (6.7)
Last number of Unprotected Coital Acts [α]	3.1 (10.7)	7.0 (17.2)	3.3 (11.0)	10.1 (21.4)	3.0 (10.4)	5.1 (13.6)
Ever recorded Unprotected Coital Acts [β]	750 (90.3)	172 (97.7)	379 (93.4)	67 (98.5)	371 (87.3)	105 (97.2)
Last Genital Ulcer Value [α]	0.19 (0.42)	0.46 (0.61)	0.17 (0.40)	0.51 (0.63)	0.21 (0.44)	0.43 (0.60)
Ever recorded Genital Ulcers [β]	537 (64.3)	139 (79.0)	262 (64.1)	57 (83.8)	275 (64.6)	82 (75.9)
Lusaka with all Time Points	n=2376	n=339	n=1305	n=147	n=1071	n=192
Donor's Age [α]	31.6 (7.9)	30.5 (7.6)	28.6 (6.6)	26.6 (6.0)	35.3 (7.7)	33.4 (7.3)
Recipient's Age [α]	32.2 (8.5)	29.0 (7.6)	35.2 (8.4)	32.7 (7.8)	28.6 (7.1)	26.2 (6.1)
Average Couple's Age [α]	31.9 (7.0)	29.7 (6.4)	31.9 (7.1)	29.7 (6.4)	31.9 (7.0)	29.8 (6.3)
Last number of Unprotected Coital Acts [α]	2.4 (8.4)	6.1 (16.4)	2.7 (8.8)	8.6 (21.3)	2.2 (7.9)	4.2 (11.0)
Ever recorded Unprotected Coital Acts [β]	1694 (75.9)	293 (88.0)	966 (78.9)	130 (90.3)	728 (72.4)	163 (86.2)
Last Genital Ulcer Value [α]	0.09 (0.31)	0.32 (0.54)	0.09 (0.29)	0.32 (0.55)	0.11 (0.33)	0.32 (0.54)
Ever recorded Genital Ulcers [β]	866 (36.5)	197 (58.1)	441 (33.8)	84 (57.1)	425 (39.7)	113 (58.9)
All Countries and all Time Points	n=4700	n=465	n=2536	n=210	n=2164	n=255
Donor's Age [α]	32.2 (7.9)	30.8 (7.8)	29.0 (6.5)	27.2 (6.4)	35.9 (7.8)	33.7 (7.6)
Recipient's Age [α]	32.3 (8.5)	29.6 (8.1)	35.2 (8.7)	33.3 (8.5)	28.9 (6.8)	26.6 (6.3)
Average Couple's Age [α]	32.3 (6.9)	30.2 (6.6)	32.1 (7.0)	30.3 (6.8)	32.4 (6.7)	30.1 (6.5)
Last number of Unprotected Coital Acts [α]	1.6 (6.5)	5.2 (14.7)	1.7 (6.8)	7.0 (18.9)	1.4 (6.2)	3.7 (9.9)
Ever recorded Unprotected Coital Acts [β]	2880 (68.2)	368 (86.4)	1643 (72.2)	168 (88.4)	1237 (63.5)	200 (84.8)
Last Genital Ulcer Value [α]	0.07 (0.26)	0.27 (0.51)	0.06 (0.25)	0.28 (0.52)	0.07 (0.27)	0.26 (0.50)
Ever recorded Genital Ulcers [β]	1381 (29.5)	235 (51.2)	719 (28.5)	106 (50.7)	662 (30.7)	129 (51.6)

[α] Average (Standard Deviation)

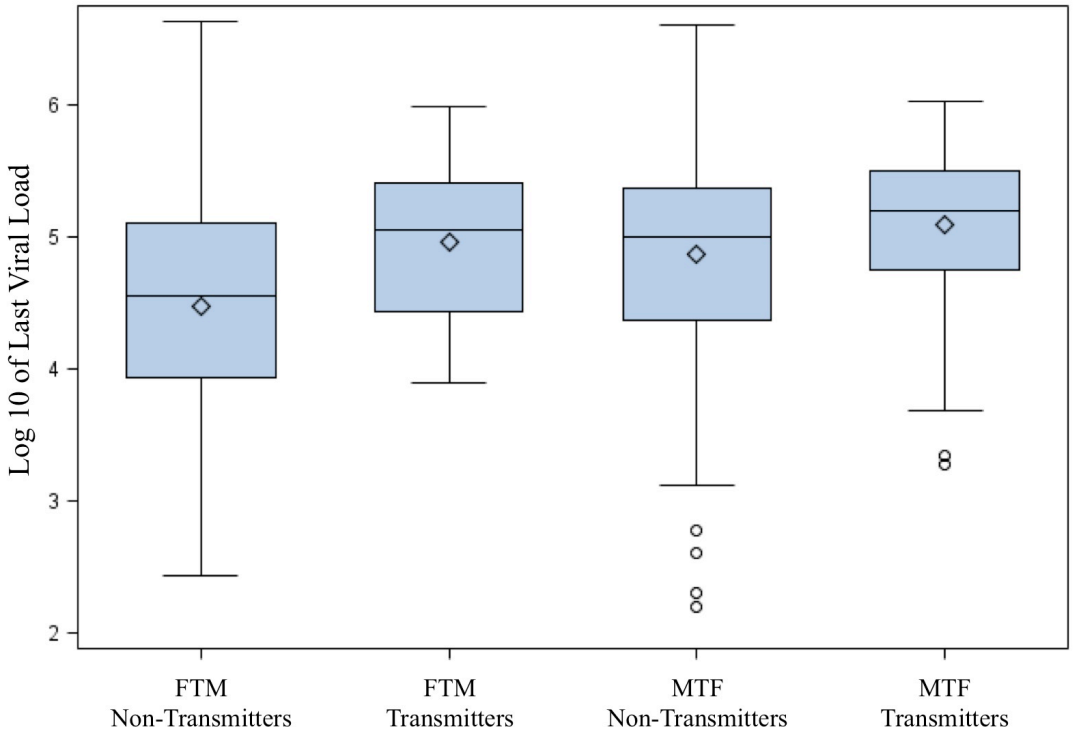
Table 1b.

	Overall				Female-to-Male Transmission				Male-to-Female Transmission			
	Non-Transmitting Couples		Transmitting Couples		Non-Transmitting Couples		Transmitting Couples		Non-Transmitting Couples		Transmitting Couples	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Lusaka with Fideli Time Points												
Ever recorded Genital Ulcers [β]	324 (40.1)	370 (46.0)	105 (61.4)	90 (52.6)	117 (28.7)	224 (55.3)	38 (55.9)	43 (63.2)	207 (51.6)	146 (36.6)	67 (65.1)	47 (45.6)
Lusaka with all Time Points												
Ever recorded Genital Ulcers [β]	554 (24.0)	502 (21.9)	154 (46.4)	111 (33.4)	229 (17.6)	316 (24.4)	60 (40.8)	55 (37.4)	325 (32.3)	186 (18.6)	94 (50.8)	56 (30.3)
All Countries and all Time Points												
Ever recorded Genital Ulcers [β]	827 (18.5)	795 (17.6)	178 (40.6)	134 (30.0)	335 (13.4)	520 (20.7)	72 (35.5)	72 (34.5)	492 (24.8)	275 (13.7)	106 (44.9)	62 (26.2)

[α] Average (Standard Deviation)
[β] Total Non-Missing (Percent)

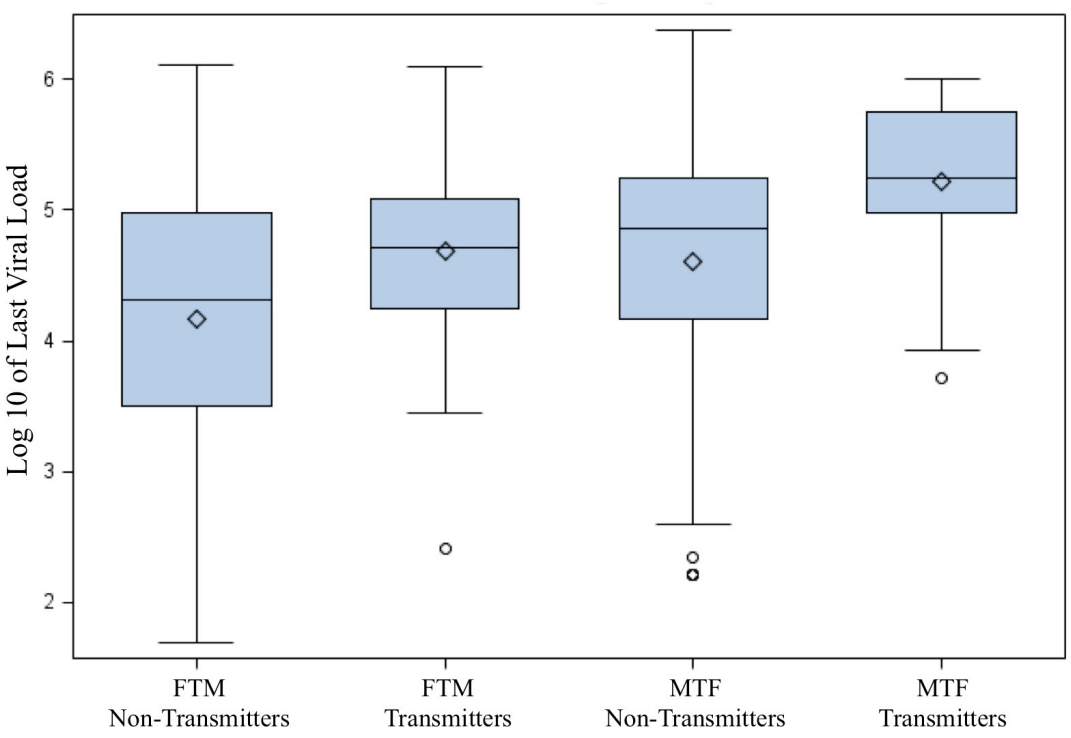
Table 2.
Hazard Ratios for model parameters are shown for an overall model, a female-to-male model, and a male-to-female model. The first data frame includes only couples recruited during the Fideli publication's time frame, February 1995 and September 2000. The second data frame includes only couples recruited at the Lusaka site in Zambia between February 1995 and August 2011. The final data frame includes data from both countries at all time points. The Ndola and Lusaka sites in Zambia are defined by the variable country=1. The Kigali site in Rwanda is defined as Country=0. The Wald Statistic and corresponding p-value under a chi square distribution with 1 degree of freedom represent a statistical difference between model estimates for female-to-male transmission and male-to-female transmission. Statistically significant differences with a p-value less than 0.05 are shown in bold.

	Overall	**FTM Transmission**	**MTF Transmission**	**Gender Difference Statistics**	
	Hazard Ratio (95% CI)	Hazard Ratio (95% CI)	Hazard Ratio (95% CI)	Wald Statistic	P-Value
Lusaka with Fideli Time Points					
Gender	0.81 (0.59, 1.11)				
Log10 of Viral Load	1.50 (1.23, 1.84)	2.35 (1.67, 3.31)	1.29 (0.99, 1.67)	**7.47**	**0.006**
Couple's Average Age	0.96 (0.94, 0.98)	0.96 (0.92, 1.00)	0.96 (0.93, 1.00)	0.04	0.838
Ulcer Information	2.20 (1.71, 2.84)	2.69 (1.83, 3.94)	1.85 (1.32, 2.61)	2.01	0.157
Unprotected Coital Acts	1.01 (1.01, 1.02)	1.01 (1.00, 1.02)	1.01 (1.00, 1.02)	0.39	0.535
Lusaka with all Time Points					
Gender	0.79 (0.63, 0.99)				
Log10 of Viral Load	1.61 (1.39, 1.86)	1.63 (1.32, 2.00)	1.40 (1.16, 1.70)	1.04	0.309
Couple's Average Age	0.96 (0.95, 0.98)	0.96 (0.94, 0.99)	0.96 (0.94, 0.99)	0.01	0.926
Ulcer Information	2.35 (1.92, 2.88)	2.79 (2.05, 3.80)	2.11 (1.60, 2.77)	1.79	0.180
Unprotected Coital Acts	1.01 (1.01, 1.02)	1.01 (1.01, 1.02)	1.01 (1.00, 1.02)	0.78	0.376
All Countries and all Time Points					
Gender	0.82 (0.67, 0.99)				
Log10 of Viral Load	1.45 (1.29, 1.62)	1.45 (1.23, 1.71)	1.47 (1.25, 1.74)	0.01	0.907
Couple's Average Age	0.96 (0.95, 0.98)	0.97 (0.95, 0.99)	0.95 (0.93, 0.98)	0.89	0.345
Ulcer Information	2.53 (2.09, 3.05)	3.14 (2.38, 4.15)	2.11 (1.62, 2.74)	**4.23**	**0.040**
Unprotected Coital Acts	1.01 (1.01, 1.02)	1.01 (1.01, 1.02)	1.01 (1.00, 1.02)	0.78	0.376
Country	2.13 (1.65, 2.74)	1.62 (1.15, 2.30)	2.76 (1.89, 4.04)	**4.10**	**0.043**



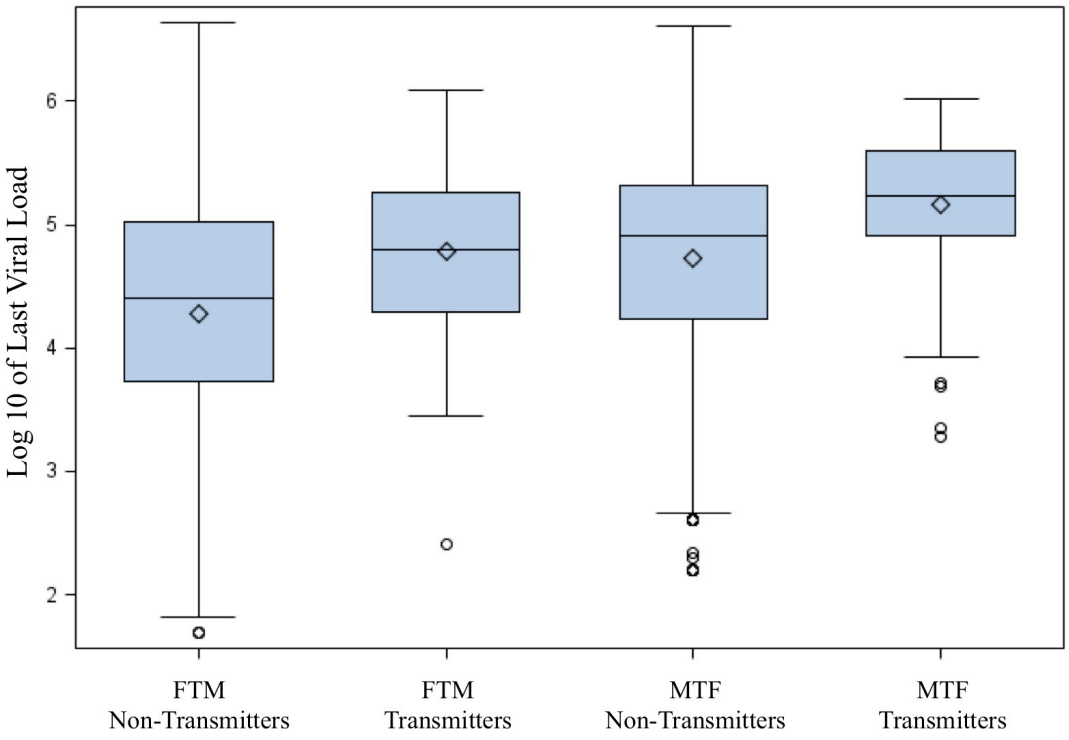
Plasma RNA value (copies/ml)	Non-Transmitting Women		Transmitting Women		Non-Transmitting Men		Transmitting Men	
Fideli Publication	**(n=93)**	**%**	**(n=41)**	**%**	**(n=114)**	**%**	**(n=63)**	**%**
(HIGH) ≥ 100,000	23	25%	22	54%	52	46%	36	57%
(MEDIUM) < 100,000 and ≥ 10,000	38	41%	16	39%	46	40%	22	35%
(LOW) < 10,000	32	34%	3	7%	16	14%	5	8%
Data Pre- Sept 1, 2000	**(n=161)**	**%**	**(n=32)**	**%**	**(n=153)**	**%**	**(n=54)**	**%**
(HIGH) ≥ 100,000	47	29%	16	50%	77	50%	35	65%
(MEDIUM) < 100,000 and ≥ 10,000	68	42%	13	41%	53	35%	16	30%
(LOW) < 10,000	46	29%	3	9%	23	15%	3	6%

Figure 1.
The table indicates the number of couples used in the Fideli publication 12 years prior and number of Lusaka couples enrolled prior to September 1, 2000 with viral loads used to make the box plots above. Data indicates plasma HIV RNA levels (copies/ml) of males and females stratified by transmission status. Mean and median is indicated by the diamond and line contained within each box respectively. The upper and lower limits of the box is bound by the interquartile range (IQR) extending from the value of the 1st to the 3rd quartile. Lines extend from the bounds of the box to the min and max of each group. Circles outside of the lines indicate outlier values a distance of 1.5 times the IQR from the box.



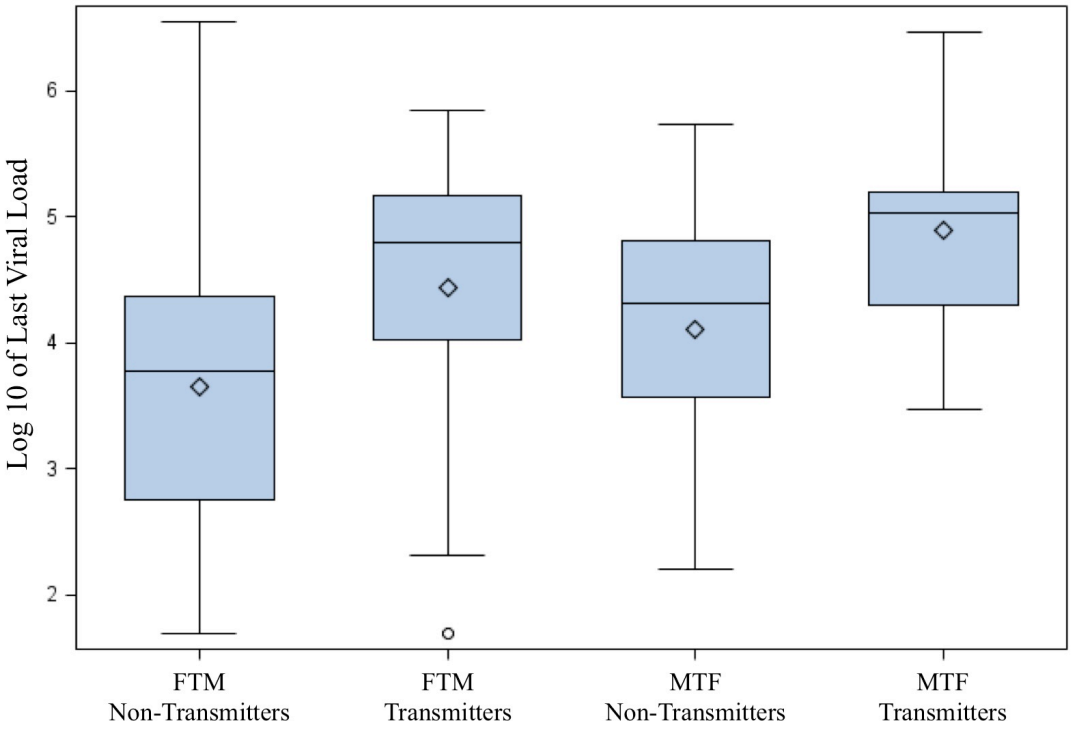
Plasma RNA value (copies/ml)	Non-Transmitting Women		Transmitting Women		Non-Transmitting Men		Transmitting Men	
	(n=260)	%	(n=60)	%	(n=192)	%	(n=68)	%
(HIGH) ≥ 100,000	61	23%	18	30%	76	40%	50	74%
(MEDIUM) < 100,000 and ≥ 10,000	100	38%	34	57%	75	39%	16	24%
(LOW) < 10,000	99	38%	8	13%	41	21%	2	3%

Figure 2.
The table indicates all Lusaka couples enrolled between after September 1, 2000 with viral loads. Data indicates plasma HIV RNA levels (copies/ml) of males and females stratified by transmission status. Mean and median is indicated by the diamond and line contained within each box respectively. The upper and lower limits of the box is bound by the interquartile range (IQR) extending from the value of the 1st to the 3rd quartile. Lines extend from the bounds of the box to the min and max of each group. Circles outside of the lines indicate outlier values a distance of 1.5 times the IQR from the box.



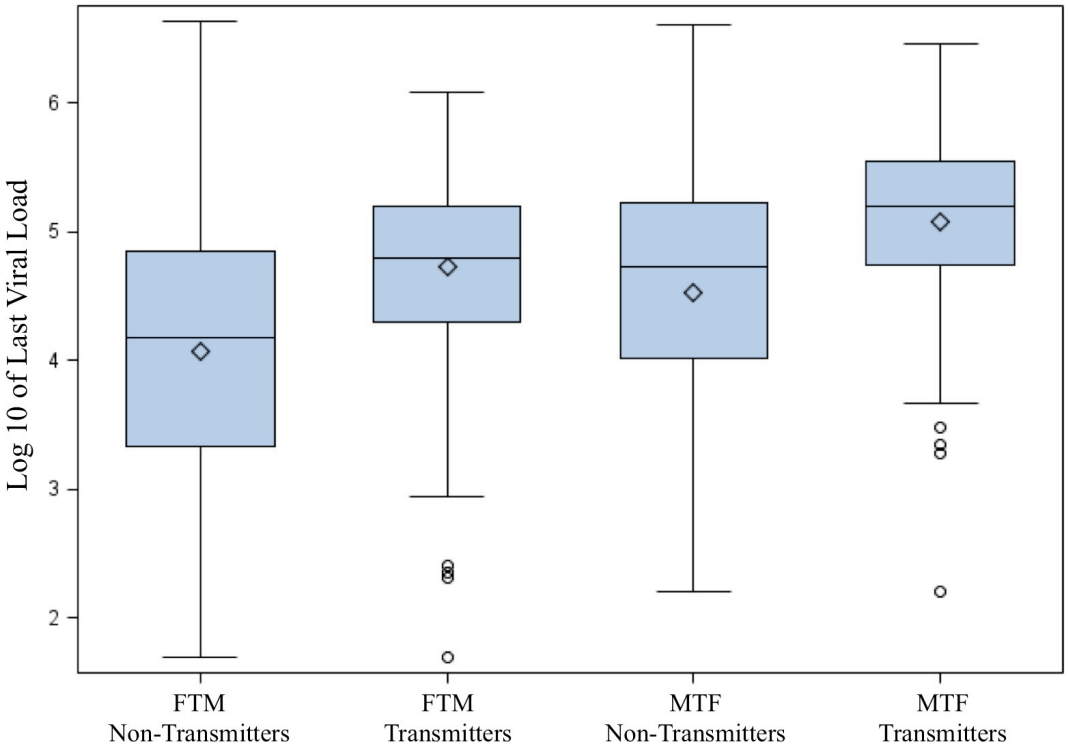
Plasma RNA value (copies/ml)	Non-Transmitting Women		Transmitting Women		Non-Transmitting Men		Transmitting Men	
	(n=421)	%	(n=92)	%	(n=345)	%	(n=122)	%
(HIGH) ≥ 100,000	108	26%	34	37%	153	44%	85	70%
(MEDIUM) < 100,000 and ≥ 10,000	168	40%	47	51%	128	37%	32	26%
(LOW) < 10,000	145	34%	11	12%	64	19%	5	4%

Figure 3.
The table indicates the number of Lusaka couples enrolled between February 1995 and January 2010 with viral loads. Data indicates plasma HIV RNA levels (copies/ml) of males and females stratified by transmission status. Mean and median is indicated by the diamond and line contained within each box respectively. The upper and lower limits of the box is bound by the interquartile range (IQR) extending from the value of the 1st to the 3rd quartile. Lines extend from the bounds of the box to the min and max of each group. Circles outside of the lines indicate outlier values a distance of 1.5 times the IQR from the box.



Plasma RNA value (copies/ml)	Non-Transmitting Women		Transmitting Women		Non-Transmitting Men		Transmitting Men	
	(n=208)	%	(n=27)	%	(n=156)	%	(n=22)	%
(HIGH) ≥ 100,000	17	8%	10	37%	27	17%	11	50%
(MEDIUM) < 100,000 and ≥ 10,000	68	33%	11	41%	68	44%	9	41%
(LOW) < 10,000	123	59%	6	22%	61	39%	2	9%

Figure 4.
The table indicates all couples from Rwanda with viral loads. Data indicates plasma HIV RNA levels (copies/ml) of males and females stratified by transmission status. Mean and median is indicated by the diamond and line contained within each box respectively. The upper and lower limits of the box is bound by the interquartile range (IQR) extending from the value of the 1st to the 3rd quartile. Lines extend from the bounds of the box to the min and max of each group. Circles outside of the lines indicate outlier values a distance of 1.5 times the IQR from the box.



Plasma RNA value (copies/ml)	Non-Transmitting Women		Transmitting Women		Non-Transmitting Men		Transmitting Men	
	(n=633)	%	(n=125)	%	(n=508)	%	(n=152)	%
(HIGH) ≥ 100,000	125	20%	47	38%	184	36%	99	65%
(MEDIUM) < 100,000 and ≥ 10,000	237	37%	61	49%	198	39%	43	28%
(LOW) < 10,000	271	43%	17	14%	126	25%	10	7%

Figure 5.
The table indicates all RZHRG couples enrolled between February 1995 and August 2011 with viral loads. Data indicates plasma HIV RNA levels (copies/ml) of males and females stratified by transmission status. Mean and median is indicated by the diamond and line contained within each box respectively. The upper and lower limits of the box is bound by the interquartile range (IQR) extending from the value of the 1st to the 3rd quartile. Lines extend from the bounds of the box to the min and max of each group. Circles outside of the lines indicate outlier values a distance of 1.5 times the IQR from the box.

APPENDIX A

```
*****************************************************************
*  Emory University - Susan Allen's Epidemiology Group
*
*  Zambia-Ndola HIV discordant couple analysis
*
*****************************************************************;

options pagesize=66 linesize=120 pageno=1 missing=' ' date
FORMCHAR="|----|+|---+=|-/\<>*";

/*****************************************************************
Program: "Part 7 Final Graphs.sas"
Programmer: Chad Stegeman
Start Date:  23 March 2012
End date: 17 April 2012

Purpose: Prints out final results needed for Thesis Manuscript

Program Notes: Outputs PDF entitled Table_1.pdf,
Final_Results.pdf, and Box_Plots.pdf

Data Sources: Includes the code to create the working combined
time dataset
*****************************************************************/

%include "h:/thesis/thesis/Part 2 Working Combinedtime.sas";
%include "h:/thesis/thesis/Collin.sas";

proc freq data=combinedtime;
     table lastulcer*boxgp;
run;

proc univariate data=combinedtime;
     where site="Z";
     var lastvlload;
     class boxgp;
run;

proc univariate data=combinedtime;
     where site="R";
     var lastvlload;
     class boxgp;
run;

/*****************************************************************
Creating Table 1
*****************************************************************/
```

```
ods pdf file="&dir\documents\Table_1.pdf" style=normalprinter;
ods html file="&dir\documents\Table_1.xls";
Proc means data=combinedtime maxdec=2;
      where site="Z" and date0 le "01Sep2000"d;
      var transage recipage avgcoupleage lastunpsex; class
knownlinkage;
      Title "Fideli Overall";
run;
Proc means data=combinedtime maxdec=2;
      where site="Z" and date0 le "01Sep2000"d  and gender=1;
      var transage recipage avgcoupleage lastunpsex; class
knownlinkage;
      Title "Fideli FTM";
run;
Proc means data=combinedtime maxdec=2;
      where site="Z" and date0 le "01Sep2000"d and gender=0;
      var transage recipage avgcoupleage lastunpsex; class
knownlinkage;
      Title "Fideli MTF";
run;

Proc means data=combinedtime maxdec=2;
      where site="Z" ;
      var transage recipage avgcoupleage lastunpsex; class
knownlinkage;
      Title "Lusaka Overall";
run;

Proc means data=combinedtime maxdec=2;
      where site="Z" and gender=1;
      var transage recipage avgcoupleage lastunpsex;  class
knownlinkage;
      Title "Lusaka FTM";
run;

Proc means data=combinedtime maxdec=2;
      where site="Z" and gender=0;
      var transage recipage avgcoupleage lastunpsex; class
knownlinkage;
      Title "Lusaka MTF";
run;

Proc means data=combinedtime maxdec=2;
      var transage recipage avgcoupleage lastunpsex; class
knownlinkage;
      Title "All Overall";
run;
```

```
Proc means data=combinedtime maxdec=2;
      where gender=1;
      var transage recipage avgcoupleage lastunpsex;  class
knownlinkage;
      Title "All FTM";
run;

Proc means data=combinedtime maxdec=2;
      where gender=0;
      var transage recipage avgcoupleage lastunpsex; class
knownlinkage;
      Title "All MTF";
run;

proc freq data=combinedtime;
      where site="Z" and date0 le "01Sep2000"d;
      table knownlinkage everunpsex*knownlinkage
everulcer*knownlinkage/norow nopercent;
      title "Fideli Overall";
run;

proc freq data=combinedtime;
      where site="Z" and date0 le "01Sep2000"d and gender=1;
      table knownlinkage everunpsex*knownlinkage
everulcer*knownlinkage/norow nopercent;
      title "Fideli FTM";
run;
proc freq data=combinedtime;
      where site="Z" and date0 le "01Sep2000"d and gender=0;
      table knownlinkage everunpsex*knownlinkage
everulcer*knownlinkage/norow nopercent;
      title "Fideli MTF";
run;

proc freq data=combinedtime;
      where site="Z" ;
      table knownlinkage everunpsex*knownlinkage
everulcer*knownlinkage/norow nopercent;
      title "Lusaka Overall";
run;

proc freq data=combinedtime;
      where site="Z" and gender=1;
      table knownlinkage everunpsex*knownlinkage
everulcer*knownlinkage/norow nopercent;
      title "Lusaka FTM";
run;

proc freq data=combinedtime;
      where site="Z" and gender=0;
      table knownlinkage everunpsex*knownlinkage
everulcer*knownlinkage/norow nopercent;
```

```
      title "Lusaka MTF";
run;

proc freq data=combinedtime;
      table knownlinkage everunpsex*knownlinkage
everulcer*knownlinkage/norow nopercent;
      title "All Overall";
run;

proc freq data=combinedtime;
      where gender=1;
      table knownlinkage everunpsex*knownlinkage
everulcer*knownlinkage/norow nopercent;
      title "All FTM";
run;

proc freq data=combinedtime;
      where  gender=0;
      table knownlinkage everunpsex*knownlinkage
everulcer*knownlinkage/norow nopercent;
      title "All MTF";
run;

Proc means data=combinedtime maxdec=2;
      where site="Z" and date0 le "01Sep2000"d;
      var lastulcer; class knownlinkage;
      Title "Fideli Overall";
run;

Proc means data=combinedtime maxdec=2;
      where site="Z" and date0 le "01Sep2000"d  and gender=1;
      var lastulcer; class knownlinkage;
      Title "Fideli FTM";
run;

Proc means data=combinedtime maxdec=2;
      where site="Z" and date0 le "01Sep2000"d and gender=0;
      var lastulcer; class knownlinkage;
      Title "Fideli MTF";
run;

Proc means data=combinedtime maxdec=2;
      where site="Z" ;
      var lastulcer; class knownlinkage;
      Title "Lusaka Overall";
run;

Proc means data=combinedtime maxdec=2;
      where site="Z" and gender=1;
      var lastulcer;  class knownlinkage;
      Title "Lusaka FTM";
run;
```

```
Proc means data=combinedtime maxdec=2;
      where site="Z" and gender=0;
      var lastulcer; class knownlinkage;
      Title "Lusaka MTF";
run;

Proc means data=combinedtime maxdec=2;
      var lastulcer; class knownlinkage;
      Title "All Overall";
run;
Proc means data=combinedtime maxdec=2;
      where gender=1;
      var lastulcer;  class knownlinkage;
      Title "All FTM";
run;
Proc means data=combinedtime maxdec=2;
      where gender=0;
      var lastulcer; class knownlinkage;
      Title "All MTF";
run;


proc freq data=combinedtime;
      where site="Z" and date0 le "01Sep2000"d;
      table mulcermax*knownlinkage fulcermax*knownlinkage/norow
nopercent;
      title "Fideli Overall";
run;
proc freq data=combinedtime;
      where site="Z" and date0 le "01Sep2000"d and gender=1;
      table mulcermax*knownlinkage fulcermax*knownlinkage/norow
nopercent;
      title "Fideli FTM";
run;
proc freq data=combinedtime;
      where site="Z" and date0 le "01Sep2000"d and gender=0;
      table mulcermax*knownlinkage fulcermax*knownlinkage/norow
nopercent;
      title "Fideli MTF";
run;

proc freq data=combinedtime;
      where site="Z" ;
      table mulcermax*knownlinkage fulcermax*knownlinkage/norow
nopercent;
      title "Lusaka Overall";
run;
proc freq data=combinedtime;
      where site="Z" and gender=1;
      table mulcermax*knownlinkage fulcermax*knownlinkage/norow
nopercent;
```

```
      title "Lusaka FTM";
run;
proc freq data=combinedtime;
      where site="Z" and gender=0;
      table mulcermax*knownlinkage fulcermax*knownlinkage/norow
nopercent;
      title "Lusaka MTF";
run;

proc freq data=combinedtime;
      table mulcermax*knownlinkage fulcermax*knownlinkage/norow
nopercent;
      title "All Overall";
run;
proc freq data=combinedtime;
      where gender=1;
      table mulcermax*knownlinkage fulcermax*knownlinkage/norow
nopercent;
      title "All FTM";
run;
proc freq data=combinedtime;
      where  gender=0;
      table mulcermax*knownlinkage fulcermax*knownlinkage/norow
nopercent;
      title "All MTF";
run;
ods html close;
ods pdf close;




ods pdf file="&dir\documents\Final_Results.pdf"
style=normalprinter;
ods html file="&dir\documents\Final_Results.xls";

/*************************************************************
Box Plots and Figure 1-3 Stuff
*************************************************************/

Proc freq data=combinedtime;
      where missvl=0 and site="Z" and date0 le "01Sep2000"d and
gender=1;
      table lastvlloadgpfr*studystatus;
      Title "FTM";
run;

Proc freq data=combinedtime;
```

```
      where missvl=0 and site="Z" and date0 le "01Sep2000"d and
gender=0;
      table lastvlloadgpfr*studystatus;
      Title "MTF";
run;

Proc freq data=combinedtime;
      where missvl=0 and site="Z" and date0 le "01Sep2000"d;
      table lastvlloadgpfr*boxgp;
      Title "Table of Last Viral Load and Knownlinkage Group for
Lusaka at Fideli time points";
run;

Proc freq data=combinedtime;
      where missvl=0 and site="Z";
      table lastvlloadgpfr*boxgp;
      Title "Table of Last Viral Load and Knownlinkage Group for
Lusaka at all time points";
run;

Proc freq data=combinedtime ;
      where missvl=0;
      table lastvlloadgpfR*boxgp;

      Title "Table of Last Viral Load and Knownlinkage Group for
all sites and time points";
run;



ods listing sge = on;

proc sgplot data=combinedtime;
      vbox loglastvlload/ category=boxgp boxwidth=0.6;
      where site="Z" and date0 le "01Sep2000"d;
      title "Distribution of Female To Male and Male To Female
Viral Loads";
      title2 "in Lusaka and entered the study before September
2000";
run;

proc sgplot data=combinedtime;
      vbox loglastvlload/ category=boxgp boxwidth=0.6;
      where Site="Z";
      title "Distribution of Female To Male and Male To Female
Viral Loads";
      title2 "in Lusaka";
run;

proc sgplot data=combinedtime;
      vbox loglastvlload/ category=boxgp
      boxwidth=0.6;
```

```
      title "Distribution of Female To Male and Male To Female
Viral Loads";
run;


/*****************************************************************
Modeling Survival Analysis --Table 2
*****************************************************************/

proc phreg data=combinedtime;
      model studytime*knownlinkage(0)=gender logt_VLLoad
t_coupleavgage t_ulcer t_unpsex /ties=exact rl;

      where site="Z" and date0 le "01Sep2000"d;

      array time{58} time1-time58;
      array ulcer{58} ulcer1-ulcer58;
      array unpsex{58} unpsex1-unpsex58;

      if studytime=eventtime then do;
            t_unpsex=lastunpsex;
      end;

      else do i=1 to 58;
            x=time{i}-90;
            if studytime le time{i} and studytime ge x then
t_unpsex=unpsex{i};
      end;

      t_ulcer=0;
      do i=1 to 58;
            y=time{i}-90;
            z=time{i}+30;
            if studytime le z and studytime ge y then
t_ulcer=ulcer{i};
      end;

      t_coupleavgage=(transage+(studytime/365.25)+recipage+(study
time/365.25))/2;

if vltime1 ne . and studytime lt vltime1 then t_vlload=vlload1;

      if vltime1 ne . and studytime ge vltime1 then do;
            if vltime2 = . then t_vlload=vlload1;
            else if vltime2 ne . then do;
                  if studytime lt vltime2 then t_vlload=vlload1;
                  if studytime ge vltime2 then do;
                        if vltime3 = . then t_vlload=vlload2;
                        else if vltime3 ne . then do;

      if studytime lt vltime3 then t_vlload=vlload2;
      if studytime ge vltime3 then do;
```

```
            if vltime4 = . then t_vlload=vlload3;
            else if vltime4 ne . then do;
                  if studytime lt vltime4 then t_vlload=vlload3;
                  if studytime ge vltime4 then do;
                        if vltime5 = . then t_vlload=vlload4;
                        else if vltime5 ne . then do;
      if studytime lt vltime5 then t_vlload=vlload4;
      if studytime ge vltime5 then do;
            if vltime6 = . then t_vlload=vlload5;
            else if vltime6 ne . then do;
                  if studytime lt vltime6 then t_vlload=vlload5;
                  if studytime ge vltime6 then do;
                        if vltime7 = . then t_vlload=vlload6;
                        else if vltime7 ne . then do;
      if studytime lt vltime7 then t_vlload=vlload6;
      if studytime ge vltime7 then do;
            if vltime8 = . then t_vlload=vlload7;
            else if vltime8 ne . then do;
                  if studytime lt vltime8 then t_vlload=vlload7;
                  if studytime ge vltime8 then t_vlload=vlload8;
            end;
      end;
                        end;
                  end;
                                                            end;
                                                      end;
                                                end;
                                          end;
                                    end;
                               end;
                        end;
                  end;
            end;
      end;

      logt_vlload=log10(t_vlload);

      Title1 "Modeling Survival Analysis, Date0 before Sept 1,
2000";
      Title2 "Lusaka Only";
      footnote ;
      footnote2 ;

      Label       logt_vlload= "Time Dependent Transmitter's Log 10
of Viral Load Data"
                  t_coupleavgage= "Time Dependent Average age of
Couple"
                  t_ulcer= "Time dependent Ulcer information"
                  t_unpsex= "Time dependent unprotected sex act
information"
                  ;
run;
```

```
proc phreg data=combinedtime;
      model studytime*knownlinkage(0)=logt_VLLoad t_coupleavgage
t_ulcer t_unpsex /ties=exact rl;

      where site="Z" and gender=1 and date0 le "01Sep2000"d;

      array time{58} time1-time58;
      array ulcer{58} ulcer1-ulcer58;
      array unpsex{58} unpsex1-unpsex58;

      if studytime=eventtime then do;
            t_unpsex=lastunpsex;
      end;

      else do i=1 to 58;
            x=time{i}-90;
            if studytime le time{i} and studytime ge x then
t_unpsex=unpsex{i};
      end;

      t_ulcer=0;
      do i=1 to 58;
            y=time{i}-90;
            z=time{i}+30;
      if studytime le z and studytime ge y then t_ulcer=ulcer{i};
      end;

      t_coupleavgage=(transage+(studytime/365.25)+recipage+(study
time/365.25))/2;

if vltime1 ne . and studytime lt vltime1 then t_vlload=vlload1;

      if vltime1 ne . and studytime ge vltime1 then do;
            if vltime2 = . then t_vlload=vlload1;
            else if vltime2 ne . then do;
                  if studytime lt vltime2 then t_vlload=vlload1;
                  if studytime ge vltime2 then do;
                        if vltime3 = . then t_vlload=vlload2;
                        else if vltime3 ne . then do;

      if studytime lt vltime3 then t_vlload=vlload2;
      if studytime ge vltime3 then do;
            if vltime4 = . then t_vlload=vlload3;
            else if vltime4 ne . then do;
                  if studytime lt vltime4 then t_vlload=vlload3;
                  if studytime ge vltime4 then do;
                        if vltime5 = . then t_vlload=vlload4;
                        else if vltime5 ne . then do;
      if studytime lt vltime5 then t_vlload=vlload4;
      if studytime ge vltime5 then do;
```

```
            if vltime6 = . then t_vlload=vlload5;
            else if vltime6 ne . then do;
                  if studytime lt vltime6 then t_vlload=vlload5;
                  if studytime ge vltime6 then do;
                        if vltime7 = . then t_vlload=vlload6;
                        else if vltime7 ne . then do;
      if studytime lt vltime7 then t_vlload=vlload6;
      if studytime ge vltime7 then do;
            if vltime8 = . then t_vlload=vlload7;
            else if vltime8 ne . then do;
                  if studytime lt vltime8 then t_vlload=vlload7;
                  if studytime ge vltime8 then t_vlload=vlload8;
            end;
      end;
                        end;
                  end;
                                                            end;
                                                      end;
                                                end;
                                          end;
                                    end;
                              end;
                        end;
                  end;
            end;
      end;

      logt_vlload=log10(t_vlload);

      Title1 "Modeling Survival Analysis, Date0 before Sept 1,
2000";
      Title2 "Lusaka Only, Female to Male Transmission";
      footnote ;
      footnote2 ;

      Label       logt_vlload= "Time Dependent Transmitter's Log 10
of Viral Load Data"
                  t_coupleavgage= "Time Dependent Average age of
Couple"
                  t_ulcer= "Time dependent Ulcer information"
                  t_unpsex= "Time dependent unprotected sex act
information"
                  ;
run;



proc phreg data=combinedtime;
      model studytime*knownlinkage(0)=logt_VLLoad t_coupleavgage
t_ulcer t_unpsex /ties=exact rl;
```

```
      where site="Z" and gender=0 and date0 le "01Sep2000"d;

      array time{58} time1-time58;
      array ulcer{58} ulcer1-ulcer58;
      array unpsex{58} unpsex1-unpsex58;

      if studytime=eventtime then do;
            t_unpsex=lastunpsex;
      end;

      else do i=1 to 58;
            x=time{i}-90;
            if studytime le time{i} and studytime ge x then
t_unpsex=unpsex{i};
      end;

      t_ulcer=0;
      do i=1 to 58;
            y=time{i}-90;
            z=time{i}+30;
            if studytime le z and studytime ge y then
t_ulcer=ulcer{i};
      end;

      t_coupleavgage=(transage+(studytime/365.25)+recipage+(study
time/365.25))/2;

if vltime1 ne . and studytime lt vltime1 then t_vlload=vlload1;

      if vltime1 ne . and studytime ge vltime1 then do;
            if vltime2 = . then t_vlload=vlload1;
            else if vltime2 ne . then do;
                  if studytime lt vltime2 then t_vlload=vlload1;
                  if studytime ge vltime2 then do;
                        if vltime3 = . then t_vlload=vlload2;
                        else if vltime3 ne . then do;

      if studytime lt vltime3 then t_vlload=vlload2;
      if studytime ge vltime3 then do;
            if vltime4 = . then t_vlload=vlload3;
            else if vltime4 ne . then do;
                  if studytime lt vltime4 then t_vlload=vlload3;
                  if studytime ge vltime4 then do;
                        if vltime5 = . then t_vlload=vlload4;
                        else if vltime5 ne . then do;
      if studytime lt vltime5 then t_vlload=vlload4;
      if studytime ge vltime5 then do;
            if vltime6 = . then t_vlload=vlload5;
            else if vltime6 ne . then do;
                  if studytime lt vltime6 then t_vlload=vlload5;
                  if studytime ge vltime6 then do;
                        if vltime7 = . then t_vlload=vlload6;
```

```
                    else if vltime7 ne . then do;
      if studytime lt vltime7 then t_vlload=vlload6;
      if studytime ge vltime7 then do;
            if vltime8 = . then t_vlload=vlload7;
            else if vltime8 ne . then do;
                  if studytime lt vltime8 then t_vlload=vlload7;
                  if studytime ge vltime8 then t_vlload=vlload8;
            end;
      end;
                        end;
                  end;
                                                      end;
                                                end;
                                          end;
                                    end;
                              end;
                        end;
                  end;
            end;
      end;

      logt_vlload=log10(t_vlload);

      Title1 "Modeling Survival Analysis, Date0 before Sept 1,
2000";
      Title2 "Lusaka Only, Male to Female Transmission";
      footnote ;
      footnote2 ;

      Label       logt_vlload= "Time Dependent Transmitter's Log 10
of Viral Load Data"
                  t_coupleavgage= "Time Dependent Average age of
Couple"
                  t_ulcer= "Time dependent Ulcer information"
                  t_unpsex= "Time dependent unprotected sex act
information"
                  ;
run;



proc phreg data=combinedtime;
      model studytime*knownlinkage(0)=gender logt_VLLoad
t_coupleavgage t_ulcer t_unpsex /ties=exact rl;

      where site="Z";

      array time{58} time1-time58;
      array ulcer{58} ulcer1-ulcer58;
      array unpsex{58} unpsex1-unpsex58;
```

```
      if studytime=eventtime then do;
            t_unpsex=lastunpsex;
      end;

      else do i=1 to 58;
            x=time{i}-90;
            if studytime le time{i} and studytime ge x then
t_unpsex=unpsex{i};
      end;

      t_ulcer=0;
      do i=1 to 58;
            y=time{i}-90;
            z=time{i}+30;
            if studytime le z and studytime ge y then
t_ulcer=ulcer{i};
      end;

      t_coupleavgage=(transage+(studytime/365.25)+recipage+(study
time/365.25))/2;

if vltime1 ne . and studytime lt vltime1 then t_vlload=vlload1;

      if vltime1 ne . and studytime ge vltime1 then do;
            if vltime2 = . then t_vlload=vlload1;
            else if vltime2 ne . then do;
                  if studytime lt vltime2 then t_vlload=vlload1;
                  if studytime ge vltime2 then do;
                        if vltime3 = . then t_vlload=vlload2;
                        else if vltime3 ne . then do;

      if studytime lt vltime3 then t_vlload=vlload2;
      if studytime ge vltime3 then do;
            if vltime4 = . then t_vlload=vlload3;
            else if vltime4 ne . then do;
                  if studytime lt vltime4 then t_vlload=vlload3;
                  if studytime ge vltime4 then do;
                        if vltime5 = . then t_vlload=vlload4;
                        else if vltime5 ne . then do;
      if studytime lt vltime5 then t_vlload=vlload4;
      if studytime ge vltime5 then do;
            if vltime6 = . then t_vlload=vlload5;
            else if vltime6 ne . then do;
                  if studytime lt vltime6 then t_vlload=vlload5;
                  if studytime ge vltime6 then do;
                        if vltime7 = . then t_vlload=vlload6;
                        else if vltime7 ne . then do;
      if studytime lt vltime7 then t_vlload=vlload6;
      if studytime ge vltime7 then do;
            if vltime8 = . then t_vlload=vlload7;
            else if vltime8 ne . then do;
                  if studytime lt vltime8 then t_vlload=vlload7;
```