Transmission probabilities of HIV and herpes simplex virus type 2, effect of male circumcision and interaction: a longitudinal study in a township of South Africa.

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Abstract

Objectives: A synergy between HIV and HSV-2 infections has been reported in observational studies. The objectives of this study were to estimate a) the per-sex-act female-to-male transmission probabilities (FtoMTPs) of HIV and HSV-2, b) the effect of each infection on the FtoMTP of the other and c) the effect of male circumcision (MC) on these FtoMTPs.

Design: We used longitudinal data collected during the MC trial conducted in Orange Farm (South Africa).

Methods: Results were obtained by specific mathematical modeling of HIV and HSV-2 statuses of the males as functions of their sexual behavior and MC status. The model took into account an estimation of the HIV and HSV-2 statuses of each of their female partners. Confidence intervals (CI) were estimated using a bootstrap re-sampling method.

Results: The HIV and HSV-2 FtoMTPs, during an unprotected sexual contact, for an uncircumcised male, in the absence of the other virus in both partners, were 0.0047 (95% CI: 0.0014-0.017) and 0.0067 (95% CI: 0.0028-0.014), respectively. HSV-2 in either partner increased HIV FtoMTP with a relative risk (RR) of 3.0 (95% CI: 1.01-7.3). Conversely, HIV in either partner increased HSV-2 FtoMTP (RR=2.5; 95% CI: 1.1-6.3). MC significantly decreased these probabilities with RRs of 0.24 (95% CI: 0.11-0.44) and 0.59 (95% CI: 0.36-0.91), respectively.

Conclusions: This study gave the first estimates of HSV-2 per-sex-act FtoMTPs in Africa. It demonstrated a synergy between HIV and HSV-2 infections and a protective effect of MC on HSV-2 acquisition by males.

Keywords: HSV-2; HIV; male circumcision; mathematical modeling; heterosexual transmission

Introduction

Observational studies have suggested an association between HIV and herpes simplex virus type 2 (HSV-2) [1-4]. However our knowledge is limited regarding their interaction. Determining a) the transmission probabilities of HSV-2 and HIV, b) the cofactor effect of each virus on the transmission probability of the other and c) the cofactor effect of male circumcision (MC) status on these transmission probabilities will help understand the dynamics of the HSV-2 and HIV epidemics in Africa and develop targeted interventions.

Published values of female-to-male HSV-2 transmission probability per sex-act were obtained from studies investigating discordant couples, mostly from developed countries. The value of 0.00015 was found by a study conducted in the United States [5]. Another study conducted in 96 sites from the United States, Canada, Europe, Latin America and Australia found a value of 0.00035 [6].

Epidemiologic studies have also estimated the probability of female-to-male HIV-1 transmission per sex-act. A study conducted in Rakai (Uganda) estimated this probability to be 0.0011 (95% confidence interval, CI: 0.0008-0.0015) [7]. However, studies in Kenya and in Thailand, investigating sexual encounters with prostitutes, evaluated per-contact HIV-1 transmission probabilities to be around 30 to 80 times higher than the Ugandan study [8, 9]. In the context of multiple partnerships, a Kenyan prospective cohort study estimated the overall probability of female-to-male HIV-1 transmission per sex-act at 0.0063 (95% CI: 0.0035-0.0091) [10]. Recently, a systematic review estimated that the crude female-to-male transmission probability per-sex-act for developing countries was 0.0030 (95% CI: 0.0009-0.010) [11].

A synergy between HIV and HSV-2 has been observed. Epidemiologic studies have demonstrated that prevalent HSV-2 is associated with a 2- to 4-fold increased risk of HIV-1 acquisition [1-4, 12]. Several mechanisms can explain this association. The presence of other sexually transmitted infections (STIs) could enhance HIV susceptibility by breaching the epithelial barrier, recruiting HIV target cells to the genital tract, or generating a pro-inflammatory local immune milieu [13]. This has been confirmed by one study which estimated the effect of genital ulceration associated with HSV-2 infection on HIV transmission probability among HIV-1 serodiscordant couples. This study found that genital ulceration in the previous 10 months was a risk factor for HIV transmission, with per-contact risk increasing 5-fold (0.0062 vs. 0.0012) [1].

In terms of co-infections, both clinical and sub-clinical reactivations of HSV-2 are associated with the influx of activated CD4+ T cells into the genital mucosa and skin, and conversely several HSV-2 proteins are capable of reactivating a latent HIV infection [14]. These interactions appear to account for the higher titers of HIV-1 in the plasma of co-infected patients: HIV-1 is shed from genital ulcers caused by HSV-2 and viral variants of HIV-1 that arise from these ulcers can appear and persist in plasma [15]. Frequent sub-clinical episodes of HSV-2 reactivation are associated with both a higher frequency and a higher amount of HIV-1 in genital secretions [16]. Hence, since genital co-infections increase HIV levels in the genital secretions, they may be an important factor in secondary sexual transmission [13].

HIV infection is also thought to facilitate HSV-2 transmission. Outbreaks of HSV-2 are generally more severe, extensive, persistent, and invasive for those with more advanced

HIV disease [15, 17]. In fact, persistent HSV-2 infection was one of the original opportunistic infections that resulted in the identification of AIDS [18].

Three randomized controlled trials demonstrated that MC reduces the female-to-male sexual acquisition of HIV by about 60% [19-21]. A meta-analysis of observational data showed that the risk reduction of HSV-2 infection by MC was of borderline statistical significance (RR=0.88, 95% CI: 0.77-1.01) [22]. Preliminary results of the effect of MC on male HSV-2 acquisition that were observed in the Rakai and Orange Farm (South Africa) MC trials were presented in international AIDS conferences [23, 24].

The study's first objective was to estimate the per-sex-act and per-partnership female-to-male transmission probabilities (FtoMTPs) of HSV-2 and HIV. The second objective was to assess the effect of each virus on the FtoMTP of the other. The last objective was to assess the effect of MC on these FtoMTPs. This analysis was conducted using a specific mathematical modeling applied to the longitudinal data of the MC trial conducted in Orange Farm among men aged 18-24 [19]. Orange Farm is a township located close to Johannesburg in Gauteng province, an area with a high HIV prevalence [25]. Samples collected during this trial were specifically tested for HSV-2. The results of a cross-sectional study conducted in the same township [26] were used to estimate the HIV and HSV-2 statuses of each female partner of the males having participated in the MC trial.

Methods

Collection of data

The technical details of the trial have been published elsewhere [19] and only a summary will be presented in this article. Between February 2002 and July 2004, 3274 uncircumcised males, aged 18 to 24, were recruited, randomized into two groups and followed up. At baseline, HSV-2 and HIV serological statuses were ascertained and MC was offered to the intervention group. During each of the follow-up visits at 3, 12 and 21 months, MC status was assessed by a nurse through genital examination and a blood sample was taken and tested for HIV and HSV-2. Information about sexual behavior was collected, including number of partners as a function of time, number of sexual contacts with each partner, reported condom use with each partner and age of each partner. The dataset used in this study included 590 additional 21-month follow-up visits (20.0% of the total number of 21-month visits) which were not included when the analysis of the effect of MC on HIV acquisition was published because corresponding laboratory data were not available at that time.

Laboratory methods

Details of the HIV testing methods have been described in the main publication of the trial [19]. Plasma samples were tested using an HSV type 2 specific IgG assay to detect HSV-2 antibodies (Kalon HSV-2 gG2 assay; Kalon Biologicals Ltd., Aldershot, UK), according to the manufacturer's recommendations.

Data analysis

We constructed two mathematical models of HIV and HSV-2 statuses as functions of time. These models were used to estimate the FtoMTPs of HIV and HSV-2. In this article, FtoMTP is defined as the probability that a susceptible male becomes infected following a sex act or partnership with an infected female. These mathematical models considered HIV and HSV-2 statuses simultaneously. Model 1 estimated the per-sex-act FtoMTPs of HIV and HSV-2, and model 2 their per-partnership FtoMTPs. These FtoMTPs were supposed to be constant as a function of time and were estimated by fitting the HIV and HSV-2 statuses predicted by the models on the observed data using the maximum likelihood method. Each of these models took into account three types of dichotomous cofactors: the effect of each virus on the FtoMTP of the other (2 cofactors), the effect of MC on the FtoMTP of each virus (2 cofactors) and the effect of reported condom use on each of these FtoMTPs (2 cofactors).

Condom use was dichotomized as follows: a given partnership was considered protected when condom use was reported as "always" used for the partnership. Otherwise the partnership was considered not protected. All sexual contacts of a protected partnership were considered as protected, otherwise they were considered as not protected.

The model estimated the HIV and HSV-2 statuses of each female partner of the males. For this estimation, we used data from a representative sample of 476 females aged 15-49 years. These data had been collected during a cross-sectional survey conducted in the same community in the year 2004 [26]. They included age, HIV serostatus, HSV-2 serostatus obtained with the same HSV-2 assay and reported number of sexual partners in the past 12 months. HIV and HSV-2 prevalences were 25.8% and 67.7%, respectively. 24.0% of women were co-infected by the two viruses. The mean (median) number of lifetime partners was 3.4 (3). For each female partner of each male of the MC trial, we estimated the probability of being infected with HIV and/or HSV-2. This estimation was done using the age of these partners and the distribution of the HIV and HSV-2 statuses of women as a function of age

and of their reported number of sexual partners. In this manner, the more sexual partners a female had had in the past 12 months the more likely she was to be a partner of males (see Annex 1 in supporting document).

The effect of each cofactor was expressed by its relative risk (RR). The RR of any cofactor was obtained by dividing the FtoMTP in the presence of the cofactor by the FtoMTP in the absence of the cofactor. This method was applied to estimate the effect of MC and the effect of reported condom use on the FtoMTPs of HIV and HSV-2. To estimate the effect of HSV-2 infection on the FtoMTP of HIV, we considered that this FtoMTP was multiplied by the corresponding RR when only one of the partners was infected by HSV-2 and that it was multiplied by RR^2 when both partners were infected. The same method was applied to estimate the effect of HIV on the FtoMTP of HSV-2. We assumed that the effects of the cofactors were constant as a function of time.

Details of the model are given in Annex 1 (see supporting document). Regarding the FtoMTP of HIV per sex act, when all the cofactors were constant (reported condom use, MC status, HSV-2 status of partners), the model assumed that the FtoMTP of HIV after n sexual contacts ($P_{n,HIV}$) was given by the following formula:

$$P_{n,HIV} = 1 - (1 - P_{HIV})^n.$$

In this formula, P_{HIV} is the FtoMTP of HIV per sex act. The formulas for HSV-2 and for the FtoMTPs per partnership are similar. The FtoMTPs of HIV and HSV-2 as well as the six RRs were estimated in a unique simulation of model 1 for the per-sex-act FtoMTPs and of model 2 for the per-partnership FtoMTPs.

A first complementary set of analyses was performed to estimate the FtoMTPs of HIV and HSV-2 with only MC status as cofactor. It generated the FtoMTP of each infection averaged on reported condom use and on the other infection. A second complementary set of analyses was conducted to allow the comparison of the results obtained by this modeling approach with those obtained by a published survival analysis performed on the same dataset [19]. For this, we estimated the *intention-to-treat* (ITT) RR of MC on the FtoMTP of HIV by replacing circumcision status by the randomization group in the model's equations with only MC as cofactor. These analyses were then repeated with HSV-2 in order to obtain the ITT RR of MC on the FtoMTP of HSV-2.

To estimate the 95% CI of the FtoMTPs and RRs, we used the bootstrap re-sampling method with 2000 replications [27]. For each bootstrap simulation, new samples of 3274 males and 476 females were randomly selected from the MC trial data for males and from the cross-sectional survey data for females. The 95% CI were estimated by the interval between the 2.5th and 97.5th percentiles of the bootstrapped simulations. The RRs were statistically compared with 1 by estimating a corresponding two-tailed *P*-value. This *P*-value was determined using the percentile (r) corresponding to a RR of 1 by $P=2r/100$ when r≤50 and by $P=2(100-r)/100$ when r≥50. When the value 1 was out of the range given by the re-sampling method, we used $P<0.001$ (2x1/2000).

Simulations and estimations were performed using the R programming language (version 2.6.1) [28]. R scripts can be provided upon request to the corresponding author.

Results

The HSV-2 and HIV prevalences at enrolment were 5.9% (194/3274) and 4.4% (145/3274), respectively. Table 1 presents the number of new HSV-2 infections, new HIV infections, and new HSV-2/HIV co-infections observed at the end of each follow-up visit.

The per-sex-act FtoMTPs of HIV and HSV-2, for an uncircumcised and non-condom user male, in the absence of the other virus in both partners, were 0.0047 (95% CI: 0.0014-0.017) and 0.0067 (95% CI: 0.0028-0.014), respectively. The corresponding per-partnership FtoMTPs were 0.017 (95% CI: 0.0065-0.044) and 0.026 (95% CI: 0.014-0.047), respectively. For each virus, the per-partnership FtoMTP was about 4 times higher than the corresponding per-sex-act FtoMTP.

Table 2 gives the multivariate RRs of the FtoMTPs of HIV per sex act and per partnership. The effects of MC and of an HSV-2 infection in either partner were similar in both cases: MC significantly reduced the FtoMTPs of HIV whereas HSV-2 infection in either partner significantly increased the FtoMTPs of HIV. Reported condom use significantly reduced the per-partnership FtoMTP of HIV and the confidence interval of the effect of reported condom use on the per-sex-act FtoMTP was large. Table 3 gives the multivariate RRs of the FtoMTPs of HSV-2 per sex act and per partnership. The results obtained were qualitatively similar to the results obtained with HIV but quantitatively weaker.

In this longitudinal study, 50.5% of males were circumcised at the beginning of the follow-up. Table 4 presents the FtoMTPs of HIV and HSV-2 per sex act and per partnership as functions of MC status, and averaged on the other cofactors. The univariate protective effect of MC status is significant for HIV and HSV-2. The protective effect on HIV is about twice the protective effect on HSV-2. The per-sex-act and per-partnership FtoMTPs of HIV, averaged on MC, reported condom use and HSV-2 status, were 0.0088 (95% CI: 0.0061-0.013) and 0.032 (95% CI: 0.022-0.045), respectively. The corresponding values for HSV-2 were close: 0.0099 (95% CI: 0.0074-0.013) and 0.037 (95% CI: 0.028-0.048), respectively.

In the univariate ITT analysis, the effects of MC on per-sex-act and per-partnership FtoMTPs of HIV were 0.37 (95% CI: 0.19-0.71, P=0.004) and 0.40 (95% CI: 0.21-0.73, P=0.004), respectively. The corresponding values for HSV-2 showed a non-significant protective effect with RR values of 0.79 (95% CI: 0.50-1.2, P=0.32) and 0.83 (95% CI: 0.54-1.2, P=0.39), respectively.

Discussion

Using a mathematical modeling approach, this study estimated the per-sex-act and per-partnership FtoMTPs of HIV and HSV-2 among a cohort of young males in South Africa. It suggested that HSV-2 infection enhanced HIV acquisition and conversely, that HIV infection could enhance HSV-2 acquisition. Furthermore, this study provided evidence of a protective effect of MC on HSV-2 acquisition by young males.

This study has some limitations: a) we used data obtained among men aged 18 to 24, recruited for a MC trial and thus not representative of the general male population; b) condom use was collected by partnership. Thus, condom use per sex act was not directly available and had to be extrapolated; c) we cannot exclude some bias since the HIV and HSV-2 serostatuses of the female partners of each male were not directly assessed but estimated. Nevertheless, in estimating the males' exposure to these two viruses for each of their sexual partnerships, we were careful to take into account the age of their female partners and the sexual behavior by age of the females of the same community; d) we also cannot exclude bias due to misreporting of sexual behavior of males and females. However, this limitation is inherent to all studies of this type.

Our estimation of HIV FtoMTP per sex act is consistent with recent values obtained by a meta-analysis of transmission studies conducted in developing countries [11]. In particular it is consistent with the results of a recent HIV-1 per-sex-act FtoMTP estimation conducted in a Kenyan prospective cohort study in the context of multiple partnerships [10]. In contrast, our estimation of HSV-2 FtoMTP per sex act is higher than the comparable published values obtained in two studies conducted among discordant couples, mostly in developed countries [5, 6]. Several factors may explain this difference [5, 29]. Using discordant couples can create a selection bias for two reasons: a) couples having a low average FtoMTP are more likely to be discordant, b) people engaged in long-term relationships have a lower FtoMTP because HSV-2 transmission decreases as a function of the duration of the partnership [6].

This study was conducted among young men who are for the most part unmarried or not living as married. The short duration of their partnerships and the low number of sexual contacts [19, 30] may explain why per-partnership FtoMTPs were only about 4 times higher than the corresponding per-sex-act FtoMTPs for HIV and HSV-2.

In this study, which used a mathematical modeling approach, the ITT and as-treated (AT) protective effect of MC on the per-sex-act and per-partnership FtoMTPs of HIV were almost identical to the reducing effect of MC on HIV incidence estimated using a statistical approach and the same dataset [19].

In both ITT and AT analyses, we observed a reducing effect of MC on HSV-2 acquisition by males, which was significant for the AT analysis. This effect was estimated by ensuring that the effect of MC on HIV and the effect of HIV on HSV-2 were taken into account. The difference between ITT and AT analyses may be partly due to the diluting effect of crossovers. This reducing effect of MC on HSV-2 acquisition is consistent with the conclusions of a meta-analysis [22] and the results of the Rakai MC trial [24]. It provides additional evidence supporting the promotion of MC in Africa as a method to reduce the

spread of STIs such as HIV and HSV-2. This effect should be further investigated by pooling the results of the three circumcision randomized trials.

We found a significant reducing effect of reported condom use on the per-partnership FtoMTPs of HIV and HSV-2. The fact that condom use per sex act was extrapolated in addition to its possible misreporting may have contributed to the large confidence intervals found for the effect of reported condom use on the per-sex-act FtoMTPs of HIV and HSV-2.

The significant enhancing effect of male or female HSV-2 positive status on the FtoMTP of HIV, as shown in this study and in many others, is now well accepted [1-4, 12]. This study showed a significant enhancing effect of HIV status on the FtoMTP of HSV-2. Such an effect could be due to transient immunosuppression during the acute stage of HIV infection which may increase HSV-2 acquisition and/or increase HSV-2 infectiousness among HIV-infected females. It should be further investigated.

The findings of this study confirm and reinforce the interpretation of a multisite study which found that sexual behavior and prevalence levels of MC and HSV-2 were key factors in understanding the heterogeneity of the HIV epidemic in Africa [31, 32]. It appears that the interactions between HIV, HSV-2, sexual behavior and MC should all be taken into account to understand the heterogeneity of the HIV and HSV-2 epidemics in Africa.

Studying the FtoMTPs of both HIV and HSV-2, we found that cofactors such as MC and the presence of the other virus had a strong effect on these FtoMTPs. Hence, it is important for transmission studies to carefully take into account these cofactors, in order to obtain comparable results independent of the prevalence of these cofactors in the study population.

The results of this study are consistent with our current knowledge of the epidemiology of HSV-2 in Africa and the synergy between the HIV and HSV-2 epidemics in this part of the world. The fact that HSV-2 treatment (acyclovir 400 mg twice daily) does not prevent HIV acquisition [33], most likely because the current HSV-2 treatment does not eradicate HSV-2, does not disprove the facilitating effect of HSV-2 on HIV acquisition. In addition to the reducing effect of MC on HIV acquisition by males, the effect of MC on HSV-2 is another argument in favor of the roll-out of MC in African countries where most males are uncircumcised [34]. Modeling studies are needed to better understand the interactions between HIV, HSV-2, MC, sexual behavior including condom use, not only in the short term, as studied by randomized controlled trials, but also in the long term.

References:

1. Corey L, Wald A, Celum CL, Quinn TC. **The effects of herpes simplex virus-2 on HIV-1 acquisition and transmission: a review of two overlapping epidemics**. *J Acquir Immune Defic Syndr* 2004,35:435-445.
2. Wald A, Link K. **Risk of human immunodeficiency virus infection in herpes simplex virus type 2-seropositive persons: a meta-analysis**. *J Infect Dis* 2002,185:45-52.
3. Freeman EE, Weiss HA, Glynn JR, Cross PL, Whitworth JA, Hayes RJ. **Herpes simplex virus 2 infection increases HIV acquisition in men and women: systematic review and meta-analysis of longitudinal studies**. *AIDS* 2006,20:73-83.
4. Brown JM, Wald A, Hubbard A*, et al.* **Incident and prevalent herpes simplex virus type 2 infection increases risk of HIV acquisition among women in Uganda and Zimbabwe**. *AIDS* 2007,21:1515-1523.
5. Wald A, Langenberg AG, Link K*, et al.* **Effect of condoms on reducing the transmission of herpes simplex virus type 2 from men to women**. *JAMA* 2001,285:3100-3106.
6. Corey L, Wald A, Patel R*, et al.* **Once-daily valacyclovir to reduce the risk of transmission of genital herpes**. *N Engl J Med* 2004,350:11-20.
7. Gray RH, Wawer MJ, Brookmeyer R*, et al.* **Probability of HIV-1 transmission per coital act in monogamous, heterosexual, HIV-1-discordant couples in Rakai, Uganda**. *Lancet* 2001,357:1149-1153.
8. Mastro TD, Satten GA, Nopkesorn T, Sangkharomya S, Longini IM, Jr. **Probability of female-to-male transmission of HIV-1 in Thailand**. *Lancet* 1994,343:204-207.
9. Cameron DW, Simonsen JN, D'Costa LJ*, et al.* **Female to male transmission of human immunodeficiency virus type 1: risk factors for seroconversion in men**. *Lancet* 1989,2:403-407.
10. Baeten JM, Richardson BA, Lavreys L*, et al.* **Female-to-male infectivity of HIV-1 among circumcised and uncircumcised Kenyan men**. *J Infect Dis* 2005,191:546-553.
11. Boily MC, Baggaley RF, Wang L*, et al.* **The risk of HIV-1 infection per sexual contact in absence of antiretroviral therapy: a systematic review and meta-analysis of observational studies.** *17th International Society for Sexually Transmitted Diseases Research*. Seattle, Washington, USA, 29 July - 1 August 2007 2007.
12. Weiss HA, Buve A, Robinson NJ*, et al.* **The epidemiology of HSV-2 infection and its association with HIV infection in four urban African populations**. *AIDS* 2001,15:S97-108.
13. Kaul R, Pettengell C, Sheth PM*, et al.* **The genital tract immune milieu: An important determinant of HIV susceptibility and secondary transmission**. *J Reprod Immunol* 2007,27:27.
14. Corey L. **Synergistic copathogens--HIV-1 and HSV-2**. *N Engl J Med* 2007,356:854-856.
15. Schacker T, Zeh J, Hu HL, Hill E, Corey L. **Frequency of symptomatic and asymptomatic herpes simplex virus type 2 reactivations among human immunodeficiency virus-infected men**. *J Infect Dis* 1998,178:1616-1622.
16. Nagot N, Ouedraogo A, Foulongne V*, et al.* **Reduction of HIV-1 RNA levels with therapy to suppress herpes simplex virus**. *N Engl J Med* 2007,356:790-799.
17. Augenbraun M, Feldman J, Chirgwin K*, et al.* **Increased genital shedding of herpes simplex virus type 2 in HIV-seropositive women**. *Ann Intern Med* 1995,123:845-847.

18. Siegal FP, Lopez C, Hammer GS, *et al.* **Severe acquired immunodeficiency in male homosexuals, manifested by chronic perianal ulcerative herpes simplex lesions**. *N Engl J Med* 1981,305:1439-1444.

19. Auvert B, Taljaard D, Lagarde E, Sobngwi-Tambekou J, Sitta R, Puren A. **Randomized, controlled intervention trial of male circumcision for reduction of HIV infection risk: the ANRS 1265 Trial**. *PLoS Med* 2005,2:e298.

20. Gray RH, Kigozi G, Serwadda D, *et al.* **Male circumcision for HIV prevention in men in Rakai, Uganda: a randomised trial**. *Lancet* 2007,369:657-666.

21. Bailey RC, Moses S, Parker CB, *et al.* **Male circumcision for HIV prevention in young men in Kisumu, Kenya: a randomised controlled trial**. *Lancet* 2007,369:643-656.

22. Weiss HA, Thomas SL, Munabi SK, Hayes RJ. **Male circumcision and risk of syphilis, chancroid, and genital herpes: a systematic review and meta-analysis**. *Sex Transm Infect* 2006,82:101-109; discussion 110.

23. Auvert B, Puren A, Talijaard D, Sobngwi-Tambekou J, Lagarde E, Sitta R. **Can male circumcision prevent acquisition of HSV-2 infection?** *16th Biennial Meeting of the International Society for Sexually Transmitted Diseases Research*. Amsterdam, The Netherlands, July 10-13, 2005 2005.

24. Tobian A, Serwadda2 D, Quinn T, *et al.* **Trial of Male Circumcision: Prevention of HSV-2 in Men and Vaginal Infections in Female Partners, Rakai, Uganda**. *CROI*. Boston, 3-6 February, 2008; USA 2008.

25. **Report National HIV and Syphilis Prevalence Survey South Africa 2006**. In: Department of Health, Pretoria, South Africa; 2006:1-31.

26. Auvert B, Males S, Puren A, Taljaard D, Carael M, Williams B. **Can Highly Active Antiretroviral Therapy Reduce the Spread of HIV?: A Study in a Township of South Africa**. *J Acquir Immune Defic Syndr* 2004,36:613-621.

27. Carpenter J, Bithell J. **Bootstrap confidence intervals: when, which, what? A practical guide for medical statisticians**. *Stat Med* 2000,19:1141-1164.

28. Team RDC. **R: A language and environment for statistical computing**. *R Foundation for Statistical Computing, Vienna, Austria* 2005.

29. Mastro TD, Kitayaporn D. **HIV type 1 transmission probabilities: estimates from epidemiological studies**. *AIDS Res Hum Retroviruses* 1998,14:S223-227.

30. Auvert B, Ballard R, Campbell C, *et al.* **HIV infection among youth in a South African mining town is associated with herpes simplex virus-2 seropositivity and sexual behaviour**. *AIDS* 2001,15:885-898.

31. Auvert B, Buve A, Ferry B, *et al.* **Ecological and individual level analysis of risk factors for HIV infection in four urban populations in sub-Saharan Africa with different levels of HIV infection**. *AIDS* 2001,15:S15-30.

32. Buve A, Carael M, Hayes RJ, *et al.* **The multicentre study on factors determining the differential spread of HIV in four African cities: summary and conclusions**. *AIDS* 2001,15:S127-131.

33. Connie Celum C, A Wald A, J Hughes J, *et al.* **HSV-2 Suppressive Therapy for Prevention of HIV Acquisition: Results of HPTN 039**. *Abstract 31499, Presented at the 15th conference on retroviruses and opportunistic infections. Boston (USA), 3-6 February 2008* 2008.

34. **New Data on Male Circumcision and HIV Prevention: Policy and Programme Implications**. In: UNAIDS-WHO, Geneva, Switzerland.; 2007.

Table 1: Number of HSV-2 and HIV infections per follow-up visit

Follow-up visit	Number of HSV-2 infections*	Number of HIV infections*	Number of co-infections*
Month-3	21	11	1
Month-12	51	22	5
Month-21	58	40	11
Total	*130*	*73*	*17*

* New infections observed at the end of each period

Table 2: Multivariate risk factors of the female-to-male per-sex-act and per-partnership transmission probabilities of HIV

Cofactor	Relative risk (95% CI) of the per-sex-act transmission probability	Relative risk (95% CI) of the per-partnership transmission probability
Presence of HSV-2*	3.0(1.01-7.3) *P*=0.048	3.7(1.7-6.9) *P*=0.003
Circumcised male	0.24 (0.11-0.44) *P*<0.001	0.25 (0.12-0.44) *P*<0.001
Reported condom protection	0.90 (0.30-1.9) *P*=0.65	0.47 (0.17-0.92) *P*=0.021

* In one of the partners. If HSV-2 is present in both partners the relative risk (RR) becomes RR^2.

Table 3: Multivariate risk factors of the female-to-male per-sex-act and per-partnership transmission probabilities of HSV-2

Cofactor	Relative risk (95% CI) of the per-sex-act transmission probability	Relative risk (95% CI) of the per-partnership transmission probability
Presence of HIV*	2.5 (1.08-6.32) *P*=0.036	3.03 (1.6-5.3) *P*< 0.001
Circumcised male	0.59 (0.36-0.91) *P*=0.021	0.61 (0.39-0.89) *P*=0.011
Reported condom protection	1.0 (0.52-1.7) *P*=0.98	0.49 (0.26-0.80) *P*=0.005

* In one of the partners. If HIV is present in both partners the relative risk (RR) becomes RR^2.

Table 4: Univariate effect of male circumcision on the female-to-male transmission probabilities of HIV and HSV-2

Transmission type	Circumcision status	Transmission probability* (95% CI)	Relative Risk (95% CI)
HIV per sex act	Circumcised	0.0036 (0.0017-0.0067)	0.23 (0.10-0.42) *P*< 0.001
	Uncircumcised	0.016 (0.010-0.026)	1
HIV per partnership	Circumcised	0.014 (0.0079-0.030)	0.26 (0.12-0.46) *P*< 0.001
	Uncircumcised	0.054 (0.045-0.10)	1
HSV-2 per sex act	Circumcised	0.0074 (0.0049-0.0106)	0.56 (0.35-0.85) *P*=0.005
	Uncircumcised	0.013 (0.0092-0.019)	1
HSV-2 per partnership	Circumcised	0.029 (0.024-0.049)	0.60 (0.39-0.88) *P*=0.001
	Uncircumcised	0.048 (0.042-0.081)	1

*Averaged on condom use and on the cofactor effect of the other virus.

Contributions

SG.M. and B.A. contributed equally to this work. DT and AP collected the data. AP, SGM, JB, EPNN, EG and BA analyzed the data. AL, JB, CL and PL wrote some parts of the paper and edited the entire manuscript.

Annex 1

1 HIV and HSV-2 statuses of females

For females, the probability of being infected was estimated based on data from a published survey [1]. Indeed, the statuses of the males' partners are unknown. These statuses e_k (k=0, 1, 2, 3) are expressed as four configurations (states) detailed in Table 1. For each female, the covariates are age, HIV and HSV-2 statuses and reported number of sexual partners in the past 12 months. The probability of being in state e_k was estimated by a multi-class logistic regression. This probability depends on age, q and the reported number of sexual partners in the past 12 months, y. Thus, we obtain the following formula:

$$\pi_k(q, y) = \frac{e^{\alpha_k q + \beta_k y}}{\sum_{j=0}^{3} e^{\alpha_j q + \beta_j y}}.$$

The reported number of sexual partners in the past 12 months was modeled by a Poisson distribution Y, which mean λ was estimated from the survey data using the maximum likelihood method. Hence:

$$\pi_k(q) = \sum_{y=0}^{+\infty} P_r(Y = y) \times \pi_k(q, y).$$

	HIV	HSV-2
e_0	-	-
e_1	+	-
e_2	-	+
e_3	+	+

Table 1: Statuses of men and women.

2 Notations and models
2.1 Settings
The HIV and HSV-2 statuses of males are determined at recruitment $\upsilon = 0$ and at the end of each of the 3 follow-up visits $\upsilon = 1, 2, 3$.

Consider an individual i. For $\upsilon = 1, 2, 3$, consider the period between visits $\upsilon - 1$ and υ; then, for that period, the observed data consist of:
$s_i^{(\upsilon)}$, the number of partners of i, $r_i^{(\upsilon, l)}$, his number of sexual contacts with his l^{th} partner, $q_i^{(\upsilon, l)}$, the age of his l^{th} partner, $\gamma_i^{(\upsilon, l)} = 1$ denotes the use of condom with a partner (0 otherwise), and $c_i^{(\upsilon)} = 1$ denotes a circumcision (0 otherwise).

2.2 Per-sex-act transmission model
2.2.1 Notations
We define the transmission probability per sex act of a given virus as the probability that a person becomes infected after a sexual contact with a person infected with the virus.
Let:

$P(hiv, \overline{hsv2})$ be the transmission probability per sex act of HIV from a woman not infected by HSV-2 to a man not infected by HSV-2, not circumcised and not condom user.

$P(hsv2, \overline{hiv})$ be the transmission probability per sex act of HSV-2 from a woman not infected by HIV to a man not infected by HIV, not circumcised and not condom user.

$RR(1)$ be the relative risk of HIV transmission to males associated with the presence of HSV-2

$RR(2)$ be the relative risk of HSV-2 transmission to males associated with the presence of HIV

$RR(0,1)$ be the relative risk of HIV transmission to males associated with condom use

$RR(1,0)$ be the relative risk of HIV transmission to males associated with male circumcision

$RR(0,2)$ be the relative risk of HSV-2 transmission to males associated with condom use

$RR(2,0)$ be the relative risk of HSV-2 transmission to males associated with male circumcision.

2.2.2 Probabilities of transmission between two visits

We derive the expression of the transmission probability per sex act, $\tilde{P}$, for a male i with his l^{th} partner between the visits $v-1$ and v ($v = 1,2,3$):

$\tilde{P}(hiv, \overline{hsv2}, \gamma_i^{(v,l)}, c_i^{(v)}) = RR(0,1)^{\gamma_i^{(v,l)}} \times RR(1,0)^{c_i^{(v)}} \times P(hiv, \overline{hsv2})$ is the transmission probability of HIV when neither the man nor his partner is infected by HSV-2

$\tilde{P}(hiv, hsv2+, \gamma_i^{(v,l)}, c_i^{(v)}) = RR(0,1)^{\gamma_i^{(v,l)}} \times RR(1,0)^{c_i^{(v)}} \times RR(1) \times P(hiv, \overline{hsv2})$ is the transmission probability of HIV when either the male or his partner is infected by HSV-2

$\tilde{P}(hiv, hsv2++, \gamma_i^{(v,l)}, c_i^{(v)}) = RR(0,1)^{\gamma_i^{(v,l)}} \times RR(1,0)^{c_i^{(v)}} \times RR(1)^2 \times P(hiv, \overline{hsv2})$ is the transmission probability of HIV when both the male and his partner are infected by HSV-2

$\tilde{P}(hsv2, \overline{hiv}, \gamma_i^{(v,l)}, c_i^{(v)}) = RR(0,2)^{\gamma_i^{(v,l)}} \times RR(2,0)^{c_i^{(v)}} \times P(hsv2, \overline{hiv})$ is the transmission probability of HSV-2 when neither the man nor his partner is infected by HIV

$\tilde{P}(hsv, hiv+, \gamma_i^{(v,l)}, c_i^{(v)}) = RR(0,2)^{\gamma_i^{(v,l)}} \times RR(2,0)^{c_i^{(v)}} \times RR(2) \times P(hiv, hsv2)$ is the transmission probability of HSV-2 when either the male or his partner is infected by HIV

$\tilde{P}(hsv2, hiv++, \gamma_i^{(v,l)}, c_i^{(v)}) = RR(0,2)^{\gamma_i^{(v,l)}} \times RR(2,0)^{c_i^{(v)}} \times RR(2)^2 \times P(hsv2, hiv)$ is the transmission probability of HSV-2 when both the male and his partner are infected by HIV.

2.2.3 Likelihood

Let
$P = (P(hiv, \overline{hsv2}), P(hsv2, \overline{hiv}))$ and $RR = (RR(1), RR(0,1), RR(1,0), RR(2), RR(0,2), RR(2,0))$.

Let i be an individual and $\varphi(P, RR, \gamma_i^{(v,l)}, c_i^{(v)})$, the vector which coordinates are:

$1 - \tilde{P}(hiv, \overline{hsv2}, \gamma_i^{(v,l)}, c_i^{(v)})$, $1 - \tilde{P}(hsv2, \overline{hiv}, \gamma_i^{(v,l)}, c_i^{(v)})$, $1 - \tilde{P}(hiv, hsv2+, \gamma_i^{(v,l)}, c_i^{(v)})$,

$1 - \tilde{P}(hsv2, hiv+, \gamma, c)$, $1 - \tilde{P}(hiv, hsv2++, \gamma_i^{(v,l)}, c_i^{(v)})$,

and $1 - \tilde{P}(hsv2, hiv++, \gamma, c)$, respectively.

For the periods 1, 2, 3 (between visits $\upsilon-1$ and υ, $\upsilon=1, 2, 3$), the contribution to the likelihood of each individual i of the sample ($L_{\upsilon,i}(e_k,e_j)$), where e_k is the state at visit $\upsilon-1$, and e_j is the state at visit υ, $k, j = 0,1,2,3$) is obtained as follow:

$$L_{\upsilon,i}(e_0,e_0) = \prod_{j=1}^{s_i^{(\upsilon)}} pm(r_i^{(\upsilon,l)},q_i^{(\upsilon,l)},\varphi_i^{(\upsilon,l)}) ;$$

$$L_{\upsilon,i}(e_0,e_1) = \sum_{t=1}^{s_i^{(\upsilon)}}\left(\prod_{j=1}^{t-1} pm\,(r_i^{(\upsilon,l)},q_i^{(\upsilon,l)},\varphi_i^{(\upsilon,l)})\right)\times um\,(r_i^{(\upsilon,l)},q_i^{(\upsilon,l)},\varphi_i^{(\upsilon,l)})\times\left(\prod_{j=t+1}^{s_i^{(\upsilon)}} qm\,(r_i^{(\upsilon,l)},q_i^{(\upsilon,l)},\varphi_i^{(\upsilon,l)})\right)$$

$$L_{\upsilon,i}(e_0,e_2) = \sum_{t=1}^{s_i^{(\upsilon)}}\left(\prod_{j=1}^{t-1} pm(r_i^{(\upsilon,l)},q_i^{(\upsilon,l)},\varphi_i^{(\upsilon,l)})\right)\times um(r_i^{(\upsilon,l)},q_i^{(\upsilon,l)},\varphi_i^{(\upsilon,l)})\times\left(\prod_{j=t+1}^{s_i^{(\upsilon)}} qm(r_i^{(\upsilon,l)},q_i^{(\upsilon,l)},\varphi_i^{(\upsilon,l)})\right) ;$$

$$L_{\upsilon,i}(e_0,e_3) = 1 - L_{\upsilon,i}(e_0,e_0) - L_{\upsilon,i}(e_0,e_1) - L_{\upsilon,i}(e_0,e_2) ;$$

$$L_{\upsilon,i}(e_1,e_1) = \prod_{j=1}^{s_i^{(\upsilon)}} qm(r_i^{(\upsilon,l)},q_i^{(\upsilon,l)},\varphi_i^{(\upsilon,l)}) ;$$

$$L_{\upsilon,i}(e_1,e_3) = 1 - \prod_{j=1}^{s_i^{(\upsilon)}} qm(r_i^{(\upsilon,l)},q_i^{(\upsilon,l)},\varphi_i^{(\upsilon,l)}) ;$$

$$L_{\upsilon,i}(e_2,e_2) = \prod_{j=1}^{s_i^{(\upsilon)}} rm(r_i^{(\upsilon,l)},q_i^{(\upsilon,l)},\varphi_i^{(\upsilon,l)}) ;$$

$$L_{\upsilon,i}(e_2,e_3) = 1 - \prod_{j=1}^{s_i^{(\upsilon)}} rm(r_i^{(\upsilon,l)},q_i^{(\upsilon,l)},\varphi_i^{(\upsilon,l)}) ;$$

With

$$\varphi_i^{(\upsilon,j)} = \varphi(P, RR, \gamma_i^{(\upsilon,j)}, c_i^{(\upsilon)}) ,$$

and for any

$$\xi = (\xi_1,\xi_2,\xi_3,\xi_4,\xi_5,\xi_6), 0 < \xi_1,\xi_2,\xi_3,\xi_4,\xi_5,\xi_6 < 1 ,$$

r,q integers:

$$pm(r,q,\xi) = \pi_0(q) + \pi_1(q)\xi_1^r + \pi_2(q)\xi_2^r + \pi_3(q)\xi_3^r\xi_4^r ;$$

$$qm(r,q,\xi) = \pi_0(q) + \pi_1(q) + \pi_2(q)\xi_3^r\xi_4^r + \pi_3(q)\cdot\xi_6^r + \pi_2(q)(1-\xi_3)\xi_4\sum_{j=1}^r(\xi_3\xi_4)^{r-j}\xi_6^{j-1} ;$$

$$rm(r,q,\xi) = \pi_0(q) + \pi_2(q) + \pi_1(q)\xi_3^r\xi_4^r + \pi_3(q)\cdot\xi_5^r + \pi_1(q)(1-\xi_4)\xi_3\sum_{j=1}^r(\xi_3\xi_4)^{r-j}\xi_5^{j-1} ;$$

$$um(r,q,\xi) = \pi_1(q)\left(1-\xi_1^r\right) + \pi_3(q)\xi_4(1-\xi_3)\sum_{j=1}^r\xi_6^{j-1}(\xi_3\xi_4)^{r-j} ;$$

$$vm(r,q,\xi) = \pi_2(q)\left(1-\xi_2^r\right) + \pi_3(q)\xi_3(1-\xi_4)\sum_{j=1}^r\xi_5^{j-1}(\xi_3\xi_4)^{r-j} .$$

2.3 Per-partnership transmission model
2.3.1 Notation
In this setting the transmission probability refers to the transmission probability per partnership from a woman to a man not infected. Thus, the previous model for transmission probability can be used with the following convention:

$P(hiv,\overline{hsv2})$ denotes the transmission probability per partnership of HIV from a woman not infected by HSV-2 to a man not infected by HSV-2.

$P(hsv2,\overline{hiv})$ denotes the transmission probability per partnership of HSV-2 from a woman not infected by HIV to a man not infected by HIV.

2.3.2 Probabilities of transmission between two visits
When a male i is with his l^{th} partner between the visits $\upsilon-1$ and υ ($\upsilon=1,2,3$), the transmission probabilities per partnership are given by the same formulas as those of section 2.2.3.

2.3.3 Likelihood
The likelihood is obtained by setting $r_i^{(\upsilon,l)}=1$ for all $i,\upsilon,l,$ and using the formulas of section 2.2.3.