TITLE: Male circumcision significantly reduces prevalence and load of anaerobic bacteria

RUNNING TITLE: Effects of circumcision on coronal sulcus microbiome

AUTHORS: Cindy M. Liu[1,2,3], Bruce A. Hungate[4], Aaron A. R. Tobian[3], David Serwadda[5], Jacques Ravel[6], Richard Lester[1], Godfrey Kigozi[5], Maliha Aziz[1], Ronald M. Galiwango[5], Fred Nalugoda[5], Tania L. Contente-Cuomo[1], Maria J. Wawer[7], Paul Keim[1,2], Ronald H. Gray[7], and Lance B. Price[1,8*]

[1] Division of Pathogen Genomics, Translational Genomics Research Institute, Flagstaff, AZ.

[2] Center for Microbial Genetics and Genomics, Northern Arizona University, Flagstaff, AZ.

[3] Department of Pathology, School of Medicine, Johns Hopkins University, Baltimore, MD.

[4] Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ.

[5] Rakai Health Sciences Program, Entebbe, Uganda.

[6] Institute for Genome Sciences, Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD.

[7] Department of Epidemiology, Johns Hopkins University, Bloomberg School of Public Health, Baltimore, MD.

[8] Department of Environmental and Occupational Health, George Washington University, Washington, DC.

CORRESPONDING AUTHOR

Lance B. Price, Ph.D.

lprice@tgen.org

ABSTRACT

Male circumcision reduces female-to-male HIV transmission. Hypothesized mechanisms for this protective effect include decreased HIV target cell recruitment and activation due to changes in the penis microbiome. To quantify the effects of male circumcision, we compared the coronal sulcus microbiota of men from the control (n = 77) and intervention (n = 79) groups at enrollment and year-1 from a randomized-controlled trial in Rakai, Uganda. We characterized microbiota using16S rRNA gene-based qPCR and pyrosequencing; log response ratio (LRR); Bayesian classification; non-metric multidimensional scaling (nMDS); and permutational multivariate analysis of variance (PerMANOVA). These analyses revealed that circumcision profoundly altered the male genital microbiota. At baseline, men in both groups had comparable coronal sulcus microbiota; however, by year-1, circumcision decreased total bacterial loads and produced less diverse microbiota with fewer dominant taxa. This loss in biodiversity was characterized by reduced prevalence and absolute abundance of 12 anaerobic bacterial taxa. Although circumcision also increased the aerobic bacterial taxa, these changes were minor compared to the decreases in anaerobes. Characterizing microbial communities in terms of absolute abundance revealed important changes that would have been obscured by a simple relative abundance approach.

IMPORTANCE

The bacterial changes identified in this study may play an important functional role in the HIV-risk reduction conferred by male circumcision. For example, decreasing the load of specific anaerobes could reduce HIV target cell recruitment to the foreskin surface, making them less vulnerable to infection. Understanding the mechanisms that underlie the benefits of male circumcision could enable researchers to identify new intervention strategies for decreasing HIV transmission. Alternative strategies may be particularly important in populations where HIV prevalence is high, but male circumcision goes against cultural traditions.

INTRODUCTION

Male circumcision (MC) reduces the risk of HIV acquisition in men by 50-60% (2, 3, 17) and decreases the incidence and prevalence of herpes simplex virus type 2 (HSV-2)(33) and human papillomavirus (HPV) (18, 33). The impact of MC on classical bacterial STIs, such as *Chlamydia trachomatis, Neisseria gonorrhoeae, Treponema pallidum,* and *Trichomonas vaginalis* is more equivocal (16, 25, 30, 33). Women with circumcised male partners are at lower risk for STIs ranging from HPV to *Trichomonas vaginalis* (16, 35). This suggests that MC reduces risk of viral STIs in men, and STI transmission to their female partners (32).

MC is hypothesized to reduce HIV risk in men by changing the penile anatomy and by altering the genital microbiology (11). With respect to the anatomic changes, MC removes the prepuce, which decreases the number of available HIV target cells on the penis (11, 21). It remains unclear whether decreases in viral STIs post-MC contribute to HIV risk reduction. HSV-2 infection increases the risk of HIV in observational studies (6, 14), but trials aimed to reduce or control viral and classical bacterial STIs have largely failed to reduce HIV transmission (19, 20). Removal of the preputial tissue also eliminates the moist subpreputial environment, which can modify the genital bacterial communities (i.e., the microbiota) and may have broad impact on the genital microbiology (27).

Recently, genital epithelial inflammation triggered by bacterial antigens has emerged as a possible factor in increasing susceptibility of genital HIV target cells (1, 10, 21). These findings suggest that particular assemblages of bacteria, including those not among the classical bacterial STIs, could promote genital epithelial inflammation. Thus, changes in the genital bacterial microbiota could be linked to HIV acquisition.

Previously, we reported the impact of MC on the coronal sulcus bacteria and overall microbiota composition based on results from 12 men (27). However, this study lacked controls from the non-circumcision arm and relied on relative abundances. In the current study, we assessed the adjusted treatment effect of MC on the genital microbiota using absolute abundance. In addition, we applied novel analyses to enumerate the microbiota changes attributable to MC. We hypothesized that MC

would significantly decrease coronal sulcus bacterial abundance and modify the microbiota in participants randomly assigned to receive MC, but not in those who remained uncircumcised. Here, we report a study of penile coronal sulcus microbiota in 77 control and 79 intervention arm participants from the Rakai MC randomized controlled trial in Uganda.

RESULTS

Coronal sulcus bacteria in the uncircumcised penis at enrollment

Prevalence. At enrollment, men from the control and intervention groups had comparable coronal sulcus bacterial prevalence (Table 1). Prevalent genera in the uncircumcised state included those from Prevotellaceae, Veillonellaceae, Clostridiales Family XI, Actinomycetaceae, Coriobacteriaceae, and Porphyromonadaceae. Two Clostridiales OTUs without known genus (i.e., Unclassified Clostridiales Family XI) or family (i.e., Unclassified Clostridiales) were also commonly detected at enrollment (Table 1).

Relative abundance. Most coronal sulcus bacteria in uncircumcised men were present at relatively low abundances (Table 1). *Prevotella* spp. was the most relatively abundant, followed by Unclassified Clostridiales, and *Corynebacterium* spp.. Six other prevalent genera—*Peptoniphilus* spp., *Anaerococcus* spp., *Finegoldia* spp., *Murdochiella* spp., *Porphyromonas* spp., and *Lactobacillus* spp.—were found at relative abundances of approximately 5%. The remaining coronal sulcus bacteria were detected at lower than 1% (Table 1).

Male circumcision reduces coronal sulcus bacterial load

Change in bacterial load. MC significantly decreased the coronal sulcus bacterial load. At enrollment, men from the control and the intervention group had similar mean bacterial loads of 1.44 x 10^5 (SD = 3.07 x 10^5) and 2.00 x 10^5 (SD = 4.81 x 10^5) bacterial 16S rRNA gene copies per µl of coronal sulcus swab eluent, respectively. The bacterial load decreased in both groups at year-1, with a mean bacterial load of 5.66 x 10^4 (SD = 1.19 x 10^5) 16S rRNA gene copies in the uncircumcised men

and 3.77 x 10^4 (SD = 1.80 x 10^5) in the circumcised men. However, the decrease was statistically greater after MC than in the uncircumcised controls (log response ratio $p = 0.048$) (Fig. 1).

Male circumcision significantly altered prevalence of coronal sulcus bacteria

Changes in prevalence. MC significantly reduced the prevalence of 15 coronal sulcus bacteria ($p < 0.05$), of which 12 are strict anaerobes, including *Porphyromonas* spp (ΔΔPrevalence = -43.10%), *Prevotella* spp. (ΔΔPrevalence = -34.21%), *Negativicoccus* spp. (ΔΔPrevalence = -28.95%), *Dialister* spp. (ΔΔPrevalence = -30.18%), *Mobiluncus* spp. (ΔΔPrevalence = -13.69%), and six genera from Clostridiales Family XI, among others (Table 2). The reductions in anaerobe prevalence due to MC were often substantial. Yet, MC did not significantly reduce prevalence of all anaerobes; notably, *Atopobium* spp., *Sneathia* spp., and *Megasphaera* spp. showed no significant decrease post-MC.

Seven coronal sulcus bacteria increased in prevalence post-MC, and among these, five also increased in the uncircumcised men, suggesting either an effect of time or from participation in the trial. Nevertheless, the increases were consistently higher among the circumcised men, as shown by the positive ΔΔPrevalence (Table 2). The aerobic *Kocuria spp*. and the facultative anaerobic *Facklamia* spp. only increased in the circumcised men. MC also increased the prevalence of other coronal sulcus bacteria that were uncommon in the uncircumcised penis (Table S1).

Male circumcision modified coronal sulcus microbiota biodiversity and composition.

Microbiota biodiversity. MC reduced evenness of the microbiota significantly, reflecting the shift from multiple to few dominant bacteria post-circumcision (*E* treatment effect = -0.053, 95% CI = -0.101 to -0.005). MC also decreased diversity of the microbiota significantly (*D* treatment effect = -1.26, 95% CI = -2.04 to -0.52).

Microbiota composition. MC significantly modified the coronal sulcus microbiota's overall composition, altering absolute abundances of microbiota constituents and increasing microbiota homogeneity (Fig 2A-B). The microbiota changes was more marked in the circumcised men

(PerMANOVA F-statistic = 13.1, $p < 0.001$) (Fig. 2A, Fig. S1) than in the uncircumcised men over time (PerMANOVA F-statistic = 3.1, $p = 0.02$) (Fig. 2B, Fig. S1).

Circumcision significantly reduced previously abundant coronal sulcus bacteria.

MC effect size. To quantify the impact of MC on coronal sulcus bacteria, we determined the MC effect size for genera that significantly decreased or increased after MC (Table 3). *Prevotella* spp., *Porphyromonas* spp., *Fusobacterium* spp., *Finegoldia* spp., *and Peptostreptococcus* spp. decreased in both prevalence and absolute abundance. For these, the mean MC effect size ranged from -1,157 to -25,327 16S rRNA gene (Table 3). The remaining genera decreased significantly either in prevalence (n = 8) or absolute abundance (n = 2). Several other negative responders had substantial decreases in many but not all circumcised men, including Unclassified Clostridiales, *Peptoniphilus* spp., and *Murdochiella* spp, and due to the high-level of inter-individual variability, effect sizes were not statistically significant (Table 3).

In contrast, the positive responders showed a smaller but statistically significant MC effect size. On average, *Corynebacterium* spp. increased by 2,860 and *Staphylococcus* spp. by 249 16S rRNA gene copies per individual (Table 3). The third highest mean MC effect size was seen in *Helcococcus* spp. As *Helcococcus* spp. belonged to Clostridiales Family XI, its response contrasts with the broadly negative impact of MC on other Clostridiales Family XI members. Overall, the relatively larger MC effect sizes in negative responders indicate that MC primarily reduced previously abundant coronal sulcus bacteria, accompanied by minor abundance gains in other bacteria. Overall, the larger MC effect sizes in negative responders indicate that MC primarily reduced previously abundant coronal sulcus bacteria, accompanied by minor abundance gains in other bacteria.

DISCUSSION

In a randomized trial of MC, we showed that MC significantly reduced bacterial load, both by reducing prevalence and absolute abundance of many coronal sulcus bacteria. We found that the two

study groups had comparable coronal sulcus microbiota, which consisted of multiple microbiota types. Overall, MC alters the composition of the microbial community and reduces microbial biodiversity. Although we find that the microbiota changed over time in uncircumcised participants as well, the effects of MC on load, abundance, composition, and diversity were statistically significant and far greater.

The role of coronal sulcus bacteria in heterosexual HIV acquisition is unknown; however, recent studies have suggested that the non-STI genital bacteria may affect the susceptibility of foreskin HIV target cells (9, 26). Of the HIV target cell types found in the foreskin, Langerhans cells (LCs)—have been hypothesized to play a key role in mediating HIV infection (9). Proximally located to the epithelial surface, naïve, LCs bind, internalize, and degrade HIV particles; however, when activated by high HIV load, active STIs, or bacteria-associated inflammatory mediators such as lipopolysaccharide (LPS) and tumor necrosis factor alpha (TNF-α), LCs bind and present HIV particles to CD4+ T-cells (9, 10).

The natural dynamics of the circumcised coronal sulcus microbiota is unknown. However, our findings based on a sampling performed one year post-circumcision are likely to represent a persistent change. Our use of novel analysis metrics, such as the log response ratio and MC effect size permitted adjustment for the impact of time and participation in the trial, which allowed us to quantify microbiota changes attributable solely to MC.

MC has been associated with reduction of BV in female sexual partners, but the sharing of genital microbiota between sexual partners is not well understood (13, 16). In this study, we show that a subset of bacteria associated with BV decreased after MC, including *Prevotella spp.*, *Fusobacterium spp.*, and *Mobiluncus spp.*, while others such as *Gardnerella* spp*., Sneathia* spp*., Actinomyces* spp.. *Atopobium* spp*., Megasphaera* spp*., and Veillonella* spp. were not altered significantly.

MC selected for bacteria capable of surviving in the circumcised coronal sulcus microenvironment. At enrollment, we find multiple microbiota types and diverse coronal sulcus bacteria in both study groups. However, nearly all of the bacteria that decreased after MC were strict anaerobes, except for *Actinomyces* spp. and *Arcanobacterium* spp, which were facultative anaerobes. We also show

that *Helcococcus* spp. was a facultative anaerobe and the single positive responder to MC in Clostridiales Family XI(8). The large competitive advantage of a single genus from a previously diverse and abundant bacterial family illustrates the strong and functionally cohesive selective pressure exerted by MC through changes to the coronal sulcus microenvironment.

One of the largest positive responders to MC reported in this study was *Staphylococcus* spp. Although we did not attempt to perform species-level analysis in the current study, additional analysis showed that the most abundant *Staphylococcus* in the post-MC coronal sulcus include *S. haemolyticus*, *S. hominis*, *S. epidermidis*, *S. xylosus*, and their genetic near neighbors. However, it is important to note that *S. aureus* and *S. epidermidis* are common commensals on exposed human epithelial and mucosal surfaces. Thus, their increase post-MC is unlikely to significantly affect the microbiota's pathogenic potential.

In the current study, we integrated culture-independent bacterial identification, an ecological analytical framework, and a randomized-controlled study design to reveal the impact of MC on the penis microbiome. Combining bacterial quantification with parallel sequencing allowed us to depart from simple relative abundance and, instead, evaluate the impacts of MC in terms of absolute bacterial abundance. Through this lens, we showed that circumcision resulted in significant decreases in the absolute abundance of several anaerobic bacterial taxa that defined the uncircumcised penis microbiome. Currently, we know little about the role of these fastidious anaerobes in the male urogenital tract, or the broader context of human health. Future studies are required to determine if decreased anaerobic bacterial load modifies foreskin inflammation and HIV target cell susceptibility, but these changes may play a critical role in the HIV risk reduction conferred by MC.

MATERIALS AND METHODS

Study Design and Subjects. We conducted a randomized trial of MC for HIV prevention in 2004-2005 % (17). In this study, HIV-negative, uncircumcised men aged 15–49 were randomized to either immediate circumcision (intervention group) or to circumcision delayed for 24 months (control group)

as described previously (17, 33). Study participants were provided access to regular reproductive health services and followed at 6, 12 and 24 months to assess HIV and sexually transmitted infection (STI) acquisition. At each visit, clinicians collected penile swabs from the coronal sulcus as follows: Dacron swabs were pre-moistened with sterile saline, and rolled over the coronal sulcus twice in a non-traumatic fashion. The swabs were immediately placed in the AMPLICOR specimen transport medium (Roche Diagnostics, Indianapolis, IN) and stored at -80°C until analysis. The study was approved by four institutional review boards: the Science and Ethics Committee of the Uganda Virus Research Institute (Entebbe, Uganda), the HIV subcommittee of the National Council for Science and Technology (Kampala, Uganda), the Committee for Human Research at Johns Hopkins University's Bloomberg School of Public Health (Baltimore, MD), and the Western Institutional Review Board (Olympia, WA).

Sample processing. We analyzed enrollment and year-1 swabs from 77 control and 79 intervention participants, selected at random from among all married men in the trial. We processed samples from each participant in the same batch to control for inter-run variation. For each sample, we lysed 100 µl of eluted transport medium using enzyme-free chemical and mechanical lysis. We purified the lysate using Qiagen AllPrep DNA/RNA Mini Kit (Qiagen, Valencia, CA, USA) and performed DNA elution using 100 µl of Buffer EB. Additional methodological details can be found in the Supplementary File.

Bacterial load quantification and 16S rRNA gene-based pyrosequencing analysis. We quantified the bacterial load, measured as the bacterial 16S rRNA gene copy per µl of coronal sulcus swab eluent using a broad-coverage qPCR assay (24). We also generated barcoded V3-V6 amplicons using broad-coverage fusion PCR primers, which were pooled and sequenced on the Genome Sequencer FLX (Roche Applied Sciences, Branford, USA). Resultant pyrosequences were chimera-checked(12), demultiplexed, and quality-checked (5). We performed taxonomic classification using the Ribosomal Database Project Naïve Bayesian Classifier (RDP Release 10, Update 28) (7). Detailed description of the bioinformatics analyses can be found in the Supplementary File.

After stringent filtering, pyrosequencing yielded a total of 104,425 16S rRNA gene sequences from control men at enrollment and 90,560 at year-1; for the intervention group, there were 88,834 16S rRNA gene sequences at enrollment and 66,265 at year-1. These sequences represented 18 phyla, 31 classes, 49 orders, and 121 families, and 306 genera at ≥ 80% bootstrap confidence level after excluding taxonomic groups with only a single sequence detected from the full sample set. For Clostridiales and Clostridiales Family XI, many sequences could not be further classified at ≥ 80% bootstrap confidence level. These were included in the dataset as Unclassified Clostridiales and Unclassified Clostridiales Family XI, respectively.

Bacterial load comparison. We expressed the bacterial load change in each participant over time as a log response ratio (LRR) using: *ln [(bacterial load at year-1)/(bacterial load at baseline)]* [Eq. 1] (23). LRR quartiles and means of participants from each group were plotted in R version 2.13.1 (31) and compared used a two-tailed *t*-test with unequal variance, at $\alpha = 0.05$.

16S rRNA gene-based microbiota comparative analysis. We analyzed the coronal sulcus microbiota based on operational taxonomic unit (OTU), i.e., the unique bacterial groups detected at each taxonomic level. We converted the per-participant OTU data into four metrics: prevalence, relative abundance, absolute abundance, and log-transformed absolute abundance.

We calculated each OTU's prevalence as: *(Total number of participants positive for the OTU in group X)/(Total number of participants in group X)* [Eq. 2] and the relative abundance as: (*Number of sequences assigned to the OTU in participant A)/(Total number of sequences from participant A)* [Eq. 3a]. We calculated absolute abundance using: *(Relative abundance of each OTU in participant A) x (Bacterial load in participant A)* [Eq. 3b] and the log-transformed absolute abundance as: *ln (Absolute abundance +1)*.

For the 40 most common genera at enrollment, we calculated the *ΔPrevalence* for each group. We compared the change in the number of individuals positive for a given genus in each study group

using two-tailed paired t-test, adjusted for false-discovery. We further assessed the change in prevalence between the two groups using $\Delta\Delta Prevalence = [(\Delta Prevalence_{intervention}) - (\Delta Prevalence_{Control})]$ [Eq. 4].

We compared the change in overall microbiota composition visually based on family-level log-transformed absolute abundance data using non-metric multidimensional scaling (nMDS) and Bray-Curtis distance (15, 22, 34). The resultant nMDS plots were annotated with centroids and 95% confidence ellipses (34). We assessed the microbiota change over time for each study group using permutational multivariate analysis of variance (PerMANOVA) (34) based on the log-transformed absolute abundance data in Euclidean distance.

We assessed the change in microbiota biodiversity in each group using two biodiversity metrics: diversity (D), calculated as D = Simpson's diversity index, and evenness (E), calculated as $E = D/S$, where S = richness (29). Evenness reflects the dominance by many (i.e., high evenness) versus few (i.e., low evenness) OTUs, whereas richness is a measurement of the total number of unique OTUs present. We calculated ΔE and ΔD for each individual and applied bootstrapping to generate random control-intervention pairs (i = 1000). We estimated the mean E and D effect sizes as: Mean $E_{E.S.} = Mean\ (\Delta E_{intervention\ Xi} - \Delta E_{control\ Yi})$ [Eq. 5a] and Mean $D_{E.S.} = Mean\ (\Delta D_{intervention\ Xi} - \Delta D_{control\ Yi})$ [Eq. 5b], as well as its accompanying 95% confidence intervals.

We identified indicator bacterial genera impacted significantly by MC with indicator species analysis using log-transformed data (28). For these indicator genera, we quantified the mean MC effect sizes [Eq. 7-8] and its 90% confidence intervals. Detailed description of the statistical analyses can be found in the Supplementary File.

Literature review. We performed literature review on the oxygen tolerance of the 40 most common genera in the uncircumcised microbiota. The Bergey's Manual of Determinative Bacteriology (4) was used for *Corynebacterium* spp., *Lactobacillus* spp., *Staphylococcus* spp., and *Streptococcus* spp. For others, we performed a search of MEDLINE via PubMed with a cutoff date of April 2012 using a

combined term of the applicable genus name and *nov* to identify publications defining new species within the genus. Detailed results from the literature review can be found in Table S2.

ACKNOWLEDGEMENTS

Funding for this work was provided by R01AI087409-01A1, U1AI51171, and 1UO1AI075115-O1A1 from the National Institutes of Health. C.M.L. was supported by the Northern Arizona University Technology and Research Initiative Fund (TRIF) fund and the Cowden Endowment in Microbiology at Northern Arizona University. A.A.R.T. was supported by the NIH 1K23AI093152-01A1 and the Doris Duke Charitable Foundation Clinician Scientist Development Award (#22006.02). The contents of this publication are solely the responsibility of the authors and do not necessarily represent the official views of the funding agencies.

REFERENCES

1. **Anderson, D., J. A. Politch, and J. Pudney.** 2011. HIV infection and immune defense of the penis. Am J Reprod Immunol **65:**220-9.
2. **Auvert, B., D. Taljaard, E. Lagarde, J. Sobngwi-Tambekou, R. Sitta, and A. Puren.** 2005. Randomized, controlled intervention trial of male circumcision for reduction of HIV infection risk: the ANRS 1265 Trial. PLoS medicine **2:**e298.
3. **Bailey, R. C., S. Moses, C. B. Parker, K. Agot, I. Maclean, J. N. Krieger, C. F. Williams, R. T. Campbell, and J. O. Ndinya-Achola.** 2007. Male circumcision for HIV prevention in young men in Kisumu, Kenya: a randomised controlled trial. Lancet **369:**643-56.
4. **Bergey, D. H., R. E. Buchanan, N. E. Gibbons, and American Society for Microbiology.** 1974. Bergey's manual of determinative bacteriology, 8th ed. Williams & Wilkins Co., Baltimore,.
5. **Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Pena, J. K. Goodrich, J. I. Gordon, G. A. Huttley, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D. McDonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsky, P. J. Turnbaugh, W. A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld, and R. Knight.** 2010. QIIME allows analysis of high-throughput community sequencing data. Nature methods **7:**335-6.
6. **Celum, C., R. Levine, M. Weaver, and A. Wald.** 2004. Genital herpes and human immunodeficiency virus: double trouble. Bull World Health Organ **82:**447-53.
7. **Cole, J. R., Q. Wang, E. Cardenas, J. Fish, B. Chai, R. J. Farris, A. S. Kulam-Syed-Mohideen, D. M. McGarrell, T. Marsh, G. M. Garrity, and J. M. Tiedje.** 2009. The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. Nucleic Acids Res **37:**D141-5.

8. **Collins, M. D., E. Falsen, K. Brownlee, and P. A. Lawson.** 2004. Helcococcus sueciensis sp. nov., isolated from a human wound. International journal of systematic and evolutionary microbiology **54:**1557-60.

9. **de Jong, M. A., and T. B. Geijtenbeek.** 2009. Human immunodeficiency virus-1 acquisition in genital mucosa: Langerhans cells as key-players. Journal of internal medicine **265:**18-28.

10. **de Witte, L., A. Nabatov, and T. B. Geijtenbeek.** 2008. Distinct roles for DC-SIGN+-dendritic cells and Langerhans cells in HIV-1 transmission. Trends in molecular medicine **14:**12-9.

11. **Dinh, M. H., K. M. Fahrbach, and T. J. Hope.** 2011. The role of the foreskin in male circumcision: an evidence-based review. Am J Reprod Immunol **65:**279-83.

12. **Edgar, R. C., B. J. Haas, J. C. Clemente, C. Quince, and R. Knight.** 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics **27:**2194-200.

13. **Eren, A. M., M. Zozaya, C. M. Taylor, S. E. Dowd, D. H. Martin, and M. J. Ferris.** 2011. Exploring the diversity of Gardnerella vaginalis in the genitourinary tract microbiota of monogamous couples through subtle nucleotide variation. PloS one **6:**e26732.

14. **Glynn, J. R., S. Biraro, and H. A. Weiss.** 2009. Herpes simplex virus type 2: a key role in HIV incidence. AIDS **23:**1595-8.

15. **Goslee, S. C. U., D.L.** 2007. The ecodist package for dissimilarity-based analysis of ecological data. Journal of Statistical Software **7:**1-19.

16. **Gray, R. H., G. Kigozi, D. Serwadda, F. Makumbi, F. Nalugoda, S. Watya, L. Moulton, M. Z. Chen, N. K. Sewankambo, N. Kiwanuka, V. Sempijja, T. Lutalo, J. Kagayii, F. Wabwire-Mangen, R. Ridzon, M. Bacon, and M. J. Wawer.** 2009. The effects of male circumcision on female partners' genital tract symptoms and vaginal infections in a randomized trial in Rakai, Uganda. Am J Obstet Gynecol **200:**42 e1-7.

17. **Gray, R. H., G. Kigozi, D. Serwadda, F. Makumbi, S. Watya, F. Nalugoda, N. Kiwanuka, L. H. Moulton, M. A. Chaudhary, M. Z. Chen, N. K. Sewankambo, F. Wabwire-Mangen, M. C. Bacon, C. F. Williams, P. Opendi, S. J. Reynolds, O. Laeyendecker, T. C. Quinn, and**

M. J. Wawer. 2007. Male circumcision for HIV prevention in men in Rakai, Uganda: a randomised trial. Lancet **369:**657-66.

18. **Gray, R. H., D. Serwadda, X. Kong, F. Makumbi, G. Kigozi, P. E. Gravitt, S. Watya, F. Nalugoda, V. Ssempijja, A. A. Tobian, N. Kiwanuka, L. H. Moulton, N. K. Sewankambo, S. J. Reynolds, T. C. Quinn, B. Iga, O. Laeyendecker, A. E. Oliver, and M. J. Wawer.** 2010. Male circumcision decreases acquisition and increases clearance of high-risk human papillomavirus in HIV-negative men: a randomized trial in Rakai, Uganda. J Infect Dis **201:**1455-62.

19. **Gray, R. H., D. Serwadda, A. A. Tobian, M. Z. Chen, F. Makumbi, T. Suntoke, G. Kigozi, F. Nalugoda, B. Iga, T. C. Quinn, L. H. Moulton, O. Laeyendecker, S. J. Reynolds, X. Kong, and M. J. Wawer.** 2009. Effects of genital ulcer disease and herpes simplex virus type 2 on the efficacy of male circumcision for HIV prevention: Analyses from the Rakai trials. PLoS Med **6:**e1000187.

20. **Gray, R. H., and M. J. Wawer.** 2008. Reassessing the hypothesis on STI control for HIV prevention. Lancet **371:**2064-5.

21. **Johnson, K. E., A. D. Redd, T. C. Quinn, A. N. Collinson-Streng, T. Cornish, X. Kong, R. Sharma, A. A. Tobian, B. Tsai, M. E. Sherman, G. Kigozi, D. Serwadda, M. J. Wawer, and R. H. Gray.** 2011. Effects of HIV-1 and herpes simplex virus type 2 infection on lymphocyte and dendritic cell density in adult foreskins from Rakai, Uganda. The Journal of infectious diseases **203:**602-9.

22. **Kindt, R. C., R. .** 2005. Tree diversity analysis, A manual and software for common statistical methods for ecological and biodiversity studies. World Agroforestry Centre (ICRAF), Nairobi.

23. **Lajeunessei, M. J.** 2011. On the meta-analysis of response ratios for studies with correlated and multi-group designs. Ecology **92:**2049-55.

24. **Liu, C. M., M. Aziz, S. Kachur, P.-R. Hsueh, Y.-T. Huang, P. Keim, and L. B. Price.** 2012. BactQuant: An enhanced broad-coverage bacterial quantitative real-time PCR assay. BMC Microbiology **12:**56.

25. **Mehta, S. D., S. Moses, K. Agot, C. Parker, J. O. Ndinya-Achola, I. Maclean, and R. C. Bailey.** 2009. Adult male circumcision does not reduce the risk of incident Neisseria gonorrhoeae, Chlamydia trachomatis, or Trichomonas vaginalis infection: results from a randomized, controlled trial in Kenya. J Infect Dis **200:**370-8.

26. **Ogawa, Y., T. Kawamura, T. Kimura, M. Ito, A. Blauvelt, and S. Shimada.** 2009. Gram-positive bacteria enhance HIV-1 susceptibility in Langerhans cells, but not in dendritic cells, via Toll-like receptor activation. Blood **113:**5157-66.

27. **Price, L. B., C. M. Liu, K. E. Johnson, M. Aziz, M. K. Lau, J. Bowers, J. Ravel, P. S. Keim, D. Serwadda, M. J. Wawer, and R. H. Gray.** 2010. The effects of circumcision on the penis microbiome. PloS one **5:**e8422.

28. **Roberts, D. W.** 2010, posting date. labdsv: Ordination and Multivariate Analysis for Ecology. [Online.]

29. **Simpson, E. H.** 1949. Measurement of Diversity. Nature **163:**688-688.

30. **Sobngwi-Tambekou, J., D. Taljaard, M. Nieuwoudt, P. Lissouba, A. Puren, and B. Auvert.** 2009. Male circumcision and Neisseria gonorrhoeae, Chlamydia trachomatis and Trichomonas vaginalis: observations after a randomised controlled trial for HIV prevention. Sex Transm Infect **85:**116-20.

31. **Team, T. R. D. C.** 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.

32. **Tobian, A. A., and R. H. Gray.** 2011. The medical benefits of male circumcision. JAMA : the journal of the American Medical Association **306:**1479-80.

33. **Tobian, A. A., D. Serwadda, T. C. Quinn, G. Kigozi, P. E. Gravitt, O. Laeyendecker, B. Charvat, V. Ssempijja, M. Riedesel, A. E. Oliver, R. G. Nowak, L. H. Moulton, M. Z. Chen,**

S. J. Reynolds, M. J. Wawer, and R. H. Gray. 2009. Male circumcision for the prevention of HSV-2 and HPV infections and syphilis. N Engl J Med **360:**1298-309.

34. **Wagner, J. O. F. G. B. R. K. P. L. R. B. O. H. G. L. S. P. S. M. H. H. S. H.** 2011. vegan: Community Ecology Package. R package version 1.17-8.

35. **Wawer, M. J., A. A. Tobian, G. Kigozi, X. Kong, P. E. Gravitt, D. Serwadda, F. Nalugoda, F. Makumbi, V. Ssempiija, N. Sewankambo, S. Watya, K. P. Eaton, A. E. Oliver, M. Z. Chen, S. J. Reynolds, T. C. Quinn, and R. H. Gray.** 2011. Effect of circumcision of HIV-negative men on transmission of human papillomavirus to HIV-negative women: a randomised trial in Rakai, Uganda. Lancet **377:**209-18.

FIGURE LEGENDS

Figure 1. Changes in mean bacterial load as measured by log response ratio, from enrollment to year-1 in the uncircumcised (*in red*) versus the circumcised men (*in orange*), as shown by group (*top panel*) and by individual (*bottom panel*). The bacterial load, as measured by 16S rRNA gene copies, decreased in more circumcised men (62/79, 78.5%) than those that remained uncircumcised (51/77, 66.2%). Additionally, the decreases in bacterial load after MC were significantly greater than the decreases over time among the controls (log response ratio $p = 0.048$).

Figure 2. nMDS plots showing changes in the coronal sulci microbiota composition in the uncircumcised men (Fig. 2A) and the circumcised men (Fig. 2B) from enrollment (*in blue*) to year-1 (*in orange*). Each data point represents an individual's microbiota at a given time point. The centroids and 95% confidence ellipses for each group are as shown. Microbiota in the uncircumcised men remained relatively consistent over time (Fig. 2A). In contrast, the coronal sulcus microbiota shifted markedly after MC, showing strong separation from the uncircumcised microbiota (Fig. 2B).