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Male circumcision reduces penile HPV incidence and persistence: a randomized controlled trial in Kenya

Running title: Male circumcision reduces penile HPV

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J. S. Smith has received research grants and consultancies from BD Diagnostics and Hologic, and supply donations from Rovers and Arbor Vita over the past five years. D. Backes, H. Chakraborty, S. Moses, K. Agot, M.G. Hudgens, W. Mei, E.Rohner, and R. C. Bailey do not have a conflict of interest with this manuscript. CJLMM is minority shareholder and part-time CEO of Self-screen B.V., a spin-off company of AmsterdamUMC (location VUmc) which develops, manufactures and licenses the high-risk HPV assay and methylation marker assays for cervical cancer screening CJLMM has a very small number of shares of Qiagen and MDXHealth, has received speakers fees from GSK, Qiagen, and SPMSD/Merck, and served occasionally on the scientific advisory boards (expert meeting) of these companies.

Requirements: word count (3930, max. 4000), figures and tables (1 figure, 5 tables, max. 6), references (32, max. 100)

ABSTRACT (236 words, max. 250)

Background: Male circumcision reduces the risk of human immunodeficiency virus infection in men. We assessed the effect of male circumcision on the incidence and natural history of human papillomavirus (HPV) in a randomized clinical trial in Kisumu, Kenya.

Methods: Sexually active, 18-24-year-old men provided penile exfoliated cells for HPV DNA testing every six months for two years. HPV DNA was detected via GP5+/6+ PCR in glans/coronal sulcus and in shaft samples. HPV incidence and persistence were assessed by intent-to-treat analyses.

Results: 2,193 men participated (1,096 randomized to circumcision; 1,097 controls). HPV prevalence was 50% at baseline for both groups and dropped to 23.7% at 24 months in the circumcision group, and 41.0% in control group. Incident infection of any HPV type over 24 months was lower among men in the circumcision group than in the control group (hazard ratio [HR], 0.61; 95% confidence interval [CI], 0.52, 0.72). Clearance rate of any HPV infection over 24 months was higher in the circumcision group than in the control group (HR, 1.87; 95% CI, 1.49, 2.34). Lower HPV point-prevalence, lower HPV incidence, and higher HPV clearance in the circumcision group were observed in glans but not in shaft samples.

Conclusion: Male circumcision reduced the risk of HPV acquisition and reinfection, and increased HPV clearance in the glans.

Impact: Providing voluntary, safe, and affordable male circumcision should help reduce HPV infections in men, and consequently, HPV-associated disease in their partners.

INTRODUCTION

Infection with oncogenic types of human papillomavirus (HPV) is the major cause of invasive cervical cancer[1] and an important cause of oral, penile and anal cancers.[2,3] Men play a crucial role in the etiology of cervical, vaginal and vulvar cancers, given transmission of penile HPV infection to female sexual partners.[4] Although prophylactic HPV vaccines are available for the prevention of high-risk HPV infections,[5,6] current generation HPV vaccines are not widely available in many geographical regions[7] and do not provide protection against all high-risk HPV types.[8]

Male circumcision has been shown in randomized controlled trials (RCTs) to reduce the risk of HIV acquisition.[9–11] A second important potential benefit of male circumcision is protection against incident penile HPV infection.[12,13] Two RCTs, one in South Africa[11] and the other in Uganda[14] have shown a protective effect of male circumcision against HPV infection in HIV-negative men. However, data are needed on the effect of male circumcision on clearance of newly acquired (incident) penile HPV infections and the rate of HPV reinfections among men with previously documented HPV infections. Furthermore, information on the effect of male circumcision on anatomical site-specific infections (glans/coronal sulcus compared to shaft) over time within an RCT setting is limited. The male circumcision RCT in Rakai, Uganda, found that male circumcision reduced the 1-year HPV point prevalence in the glans/coronal sulcus and in the shaft, yet results were limited to a subset of approximately 100 participants at one cross-sectional time point.[15] In an RCT setting in Kisumu, Kenya, we have previously found evidence that male circumcision was associated with a reduced hazard of acquiring high-viral load (>250 copies/scrape) HPV-16 and HPV-18 infections in the glans, but HPV viral load results for shaft samples were weaker and less precise.[16]

Based on the same RCT study population in Kenya, we now present additional in-depth results of the effect of male circumcision on penile HPV incidence, clearance, and reinfection over two years of follow-up with penile samples collected separately from the glans/coronal sulcus and the shaft.

METHODS

Study Population, Enrollment, and Follow-up

Uncircumcised men were screened for eligibility between February 2002 and September 2005 to participate in an RCT of male circumcision (clinical trials registration number: NCT00059371).[9] The main objective of this RCT was to assess the effect of male circumcision on HIV incidence. Enrollment criteria included being uncircumcised, age 18-24 years, HIV seronegative, sexually active, having blood hemoglobin ≥90 g/L and providing signed informed consent. Participants were recruited from sexually transmitted infection (STI) clinics, workplaces, and community organizations and events in Kisumu, Kenya. Participants randomized to the intervention arm underwent male circumcision on the same day as RCT enrollment when the penile samples were collected, or as soon as possible after, mostly within a few days.[9] The majority of male circumcisions were completed on the day of randomization (64%), 80% within one day, 85% within two days, 88% within three days, and 95% within six weeks of randomization.[9] Analyses presented here are based on an ancillary HPV study nested within this male circumcision RCT. HPV testing was performed on participants consenting to collection of penile exfoliated cells, and who had a minimum of one follow-up visit.

Of 2,784 men enrolled in the main RCT,[9] 2,299 (83%) men gave consent to provide penile swab samples and had an HPV result at baseline. Of those, 2,193 (95%) had both baseline and follow-up HPV

results. Therefore, 2,193 men (uncircumcised at baseline) were included in analyses (1,096 randomized to male circumcision and 1,097 randomized to the control group). At baseline, standardized questionnaires on socio-demographic characteristics and sexual behavior were administered to participants by trained male interviewers. Penile cell, blood and urine samples were collected for testing of HPV and other STIs at baseline, 6, 12, 18, and 24 months. Most participants attended their 6-month (91%), 12-month (89%), 18-months (87%), and 24-month (86%) follow-up visits.

The protocol was approved by Institutional Review Boards of the Universities of Illinois at Chicago, Manitoba, Nairobi and North Carolina; by RTI International; and by the AmsterdamUMC, location VUmc, Amsterdam, The Netherlands.

Penile Cell Collection and Processing

Penile exfoliated cells for HPV DNA detection were collected by a trained physician or clinical officer from two anatomical sites: i) glans, coronal sulcus, and inner foreskin tissue (glans specimen); and ii) shaft and external foreskin tissue (shaft specimen), using pre-wetted Type 3 Dacron swabs.[17,18] Swabs were placed in 15-mL centrifuge tubes containing 2 mL 0.01 mol/L Tris-HCl, 7.4 pH buffer, and processed on the collection day at the research laboratory by centrifugation at 3,000g for 10 minutes. Cell pellets were resuspended in 0.1 mL of Tris-HCl buffer and frozen at -75°C. Samples were shipped in liquid nitrogen to the Department of Pathology, AmsterdamUMC, location VUmc, for HPV testing.

Type-specific HPV DNA and STI Testing

DNA was isolated from samples using the NucleoSpin 96 Tissue kit (Macherey-Nagel, Düren, Germany) and Michrolab Star robotic system (Hamilton, Martinsried, Germany) according to manufacturers' instructions. Presence of human DNA was evaluated by *β*-globin polymerase chain reaction (PCR), followed by agarose gel electrophoresis. Overall, β-globin positivity in glans and/or shaft specimens was 63.1% at baseline, 66.7% at 6 months, 77.1% at 12 months, 68.4% at 18 months, and 80.3% at 24 months. The β-globin positivity in glans was 56.7% at baseline, 57.3% at 6 months, 67.0% at 12 months, 59.8% at 18 months, and 71.9% at 24 months. The β-globin positivity in shaft was 35.2% at baseline, 36.8% at 6 months, 46.5% at 12 months, 37.2% at 18 months, and 51.0% at 24 months. Results were similar when analyses were restricted to *β*-globin-positive samples; thus, analyses utilized HPV DNA data from all penile exfoliated cell specimens, regardless of *β*-globin positivity, unless otherwise stated.

HPV DNA positivity was assessed by GP5+/6+ PCR, followed by hybridisation of PCR products using an enzyme immunoassay readout with two HPV oligoprobe cocktails that, together, detect 44 HPV types. Subsequent genotyping was performed by reverse line blot hybridization.[18–20] HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 were considered high-risk types. HPV types detected by enzyme immunoassay, but not by reverse line blot genotyping, were designated as HPVX, indicating a type, sub-type or variant not detectable by probes used in enzyme immunoassay.

At baseline, urine samples were tested for *Neisseria gonorrhoeae* (GC) and *Chlamydia trachomatis* (CT) by PCR (Roche Diagnostics) and *Trichomonas vaginalis* (TV) by culture (BioMed Diagnostics Inc.). If urethral discharge was present, urethral swab specimens were tested for GC and CT by PCR, and GC and TV by culture. If a genital ulcer was present, swabs of the ulcer were tested for *Haemophilus ducreyi* (HD) by PCR and culture. Serum was tested for herpes simplex virus type 2

(HSV-2) antibody (Kalon Biological Ltd); and for HIV antibody using two rapid tests (Determine, Abbott Diagnostic Division, and Unigold, Trinity Biotech), and confirmed by double ELISA (Adaltis Inc.; Trinity Biotech) at the University of Nairobi as previously described.[9] Positive serum Rapid Plasma Reagin (Becton, Dickinson and Company) tests for syphilis were confirmed by Treponema pallidum hemagluttination assay (Randox Laboratories Ltd).

Statistical Methods

At any fixed timepoint, men with multiple HPV type infections were considered to have high-risk HPV if one or more high-risk types were detected, and to have low-risk HPV if only low-risk types were detected. Men with untyped HPV infections (HPV-X) were excluded from analyses involving HPV-risk categorizations, unless they had a high-risk HPV type concurrently detected. Unadjusted prevalence risk ratios (PRRs) between the circumcision and control arm were estimated at each study visit, separately by anatomical site, and then overall by combining results from both anatomical sampling sites for the same man. PRRs were calculated for HPV type groupings: any, high-risk, low-risk, single, and multiple infections. HPV analyses utilized data from all penile exfoliated cell specimens regardless of β-globin positivity; sensitivity analyses were conducted restricting analyses to β-globin positive results.

Analyses were conducted to determine the effect of male circumcision on HPV prevalence, incidence, and clearance, where all men were analyzed according to their randomization assignment (intention-to-treat analysis). As-treated analyses were also conducted, which entailed including in the survival models a time-dependent covariate for circumcision status at each follow-up visit to take into account those individuals who did not adhere to their randomization assignment.[9]

An incident, or acquired, infection was defined as detection of type-specific HPV infection during follow-up that was not present at baseline. Time to incident HPV infection was estimated by assuming that infections were acquired at the midpoint between the last HPV-negative result and first subsequent HPV-positive result. Men were censored at their last visit if they remained negative for that HPV type. Incidence rates for each HPV type or HPV grouping were estimated among participants negative for the given individual HPV type or groupings at baseline. Incidence rate analyses were conducted for the most common HPV types in the glans or shaft and for HPV type groupings, stratified by anatomical site and for the two sites combined. Non-parametric estimates of the cumulative probability of any HPV infection among men who were HPV-negative at baseline were obtained using the Kaplan-Meier method, allowing for interval censored infection times.[21] Parametric survival models that allow for interval censored data were used to estimate HRs of the effect of male circumcision on time to newly acquired HPV infections.

HPV clearance was defined as a positive type-specific HPV result followed by at least one HPV-negative result for that specific HPV type. HPV clearance was considered to have occurred at the midpoint between dates of the last HPV-positive result and of the first subsequent HPV-negative result. Clearance analyses were conducted among HPV infections present at baseline. HPV infections were used as units of analysis to account for men with multiple-type infections. Non-parametric estimates of the cumulative probability of clearing an HPV infection were obtained using the Kaplan-Meier method allowing for interval censored clearance times. The effect of circumcision on HPV clearance was estimated using random effect parametric survival models that allowed for interval censored data and multiple infections per man.

Sub-analyses were conducted to examine type-specific HPV reinfections rates among men who were positive for a given type at baseline, cleared the HPV infection, and then re-acquired the same HPV type; only first repeat infections were counted. Clearance of newly acquired (incident) penile HPV infections not present at baseline were also compared between the circumcision and control groups.

RESULTS

Of 2,193 participating men, the median age was 20 years (interquartile range [IQR],19-22) at baseline. Most participants were of Luo ethnicity (98.5%), unmarried (94.1%), had secondary education (65.2%), and were unemployed (64.0%; Table 1). The percentage of men positive at baseline was 26.9% for HSV2, 4.6% for CT, 2.1% for TV, 2.2% for GC, 0.9% for syphilis, and 0% for HD (Table 1). Median age at first sexual intercourse was 16 years (IQR, 14-17) and median number of lifetime female partners was 4 (IQR, 3-7). Most (87.5%) subjects reported sexual intercourse in the last 6 months; of those, 52.7% used condoms inconsistently and 25.5% never. The two arms had similar demographic characteristics and sexual histories at baseline.

Prevalence of HPV infection

Baseline HPV prevalence was similar in men randomized to male circumcision (50.4%) and to control (49.7%; PRR, 1.01; 95% CI, 0.93, 1.10 overall; 0.99 [0.89, 1.10] in β-globin positive samples; Table 2). At six months, HPV prevalence in the circumcision group dropped to 29.8% vs. 45.3% in the control group (PRR, 0.66; 95% CI, 0.58, 0.74). At 12 months, HPV prevalence was 26.6% and 48.4% in the circumcision and the control group, respectively (PRR, 0.55; 95% CI, 0.49, 0.62 overall; 0.58 [0.51, 0.68] in β-globin positive samples). At 24 months, HPV prevalence was 23.7% and 41.0% in the

circumcision and the control group, respectively (PRR, 0.58; 95% CI, 0.50, 0.66 overall; 0.57 [0.49, 0.67] in β -globin positive samples). PRRs were similar for high-risk, low-risk, and multiple HPV infections, dropping from values near 1.0 at baseline to 0.61, 0.76, and 0.55, respectively, at 6 months and 0.55, 0.59, and 0.42, respectively, at 24 months.

Incidence of HPV Infection

The incidence of infections of any HPV type in the glans or shaft specimens over 24 months was lower in the circumcision (50.3 per 100 person-years; 95% CI, 44.3, 56.9) than the control group (75.5 per 100 person-years; 95% CI, 67.8, 84.0; HR, 0.61; 95% CI, 0.52, 0.72 overall; 0.56 [0.45, 0.70] in analyses restricted to β-globin positive samples) among those who were negative for a specific HPV type at baseline (Table 3). In this same group, rates of incident infections for high-risk HPV, low-risk HPV, HPV16/18, HPV16/18/6/11, single and multiple HPV types over 24 months were lower in the circumcision than the control group (HRs, 0.46 to 0.77). This trend was consistent when restricted to individual HPV types, albeit to different degrees (HRs, 0.37 to 0.70). Similarly, re-infection rates following baseline positivity and subsequent negativity of any HPV, high-risk HPV, single and multiple HPV types were lower in the circumcision group compared to the control group (HRs, 0.46 to 0.69), a trend that was reflected, to various degrees, by individual HPV types (HRs, 0.10 to 1.14). The HRs of reinfections of any type HPV were similar for analyses among all samples (HR, 0.66; 95% CI, 0.54, 0.81) and those restricted to β-globin positive samples (HR, 0.66; 95% CI, 0.50, 0.86; Table 3).

Among men who were HPV negative at baseline, those in the circumcision group were less likely to have an incident HPV infection detected at follow-up compared to the control group (24-month

cumulative incidence, 47.5%; 95% CI, 43.1%-51.9% versus 62.5%; 95% CI, 58.4%-66.6%; P<0.001; Figure 1A).

Clearance of HPV infection

The clearance rate of any HPV infection present at baseline over 24 months was higher in the circumcision group (272 per 100 person-years; 95% CI, 257, 289) than in the control group (212 per 100 person-years; 95% CI, 200, 225; HR, 1.87; 95% CI, 1.49, 2.34 overall; HR, 1.98; 95% CI, 1.48, 2.66 in *β*-globin positive samples; Table 4). In men with prevalent HPV infection at baseline, clearance rates of low-risk HPV, high-risk HPV, HPV16/18, HPV16/18/6/11, and multiple HPV types were all higher in the circumcision than the control group (HRs, 1.50 to 1.89). This trend was reflected by common individual HPV types (except HPV35 and 6), albeit to different degrees (HRs, 1.23 to 1.89).

Similarly, in men without the specific HPV type infection at baseline, clearance rates of any HPV, high-risk HPV, low-risk HPV, and multiple incident HPV infection acquired after baseline were higher in the circumcision than the control group (HRs, 1.48 to 1.75). This trend was reflected by common individual HPV types (except HPV6), albeit to different degrees (HRs, 1.11 to 2.22). The HR of clearance of incident infections not present at baseline among all samples (HR, 1.68; 95% CI, 1.39, 2.02) was similar to analyses restricted to *β*-globin positive samples (HR, 1.63; 95% CI, 1.26, 2.10; Table 4).

Among HPV infections present at baseline, the estimated time to clearance was less among men in the circumcision arm (79.7% estimated probability of clearing infection by 6 months) compared to men in the control arm (51.5% estimated probability of clearing infection by 6 months; Figure 1B).

HPV Infection by Anatomical Site

HPV prevalence was consistently higher in the glans than shaft for all HPV groupings (e.g. overall HPV prevalence at baseline in controls: 46% in glans; 17% in shaft; Table 5). In the glans, HPV prevalence was lower in the circumcision than in the control arm at all post-baseline visits and for all HPV-type groupings post-baseline (PRRs, 0.31 to 0.72), while in the shaft, no differences were observed in HPV prevalence between the circumcision and the control arm.

Men in the circumcision group had lower incidence rates (Supplementary Table 1) and higher clearance rates (Supplementary Table 2) of HPV infection in glans samples, but not in shaft samples, although several point estimates were not reliable for individual types due to relatively small sample sizes within strata.

There were lower reinfection rates for all HPV type groupings in the circumcision compared to the control arm in the glans (HRs, 0.41 to 0.88); and for any HPV, high-risk HPV, and low-risk HPV (HRs, 0.65 to 0.71) in the shaft (Supplementary Table 3). We also observed higher HPV clearance among men with new, incident HPV infections in the circumcision group for the glans (any HPV, high-risk HPV, low-risk HPV, HPV16, and HPV56; Supplementary Table 4). Associations were relatively imprecise for both of these sub-analyses, particularly for individual HPV types.

As-treated analyses

Results for the as-treated analysis were similar to the intent to treat results. In particular, for incident infections of any HPV type over 24 months the hazard was lower when men were circumcised (HR 0.58; 95% CI 0.49, 0.69). Likewise, the clearance rate of any HPV infection over 24 months was higher when men were circumcised (HR 1.50; 95% CI 1.39, 1.62)

DISCUSSION

In this large RCT of male circumcision and HPV infection, men in the circumcision group had approximately 40% lower incidence and 35% lower HPV reinfection rate over 24 months than the control group. Men in the circumcision group had an approximately 40% lower prevalence of overall, high-risk, and low-risk HPV infections for combined glans and shaft specimens than the control group at all post-baseline visits, with prevalence decreasing notably from the baseline to the six month visit and remaining relatively stable over time from 12 to 24 months. Male circumcision was associated with at least 50% higher clearance of any, high-risk, and low-risk prevalent HPV infections over 24 months, and similar clearance of newly acquired HPV infections in combined glans/shaft specimens as compared with the control group. Male circumcision was most strongly associated with lower incidence and higher clearance rates of multiple HPV type infections, with similar findings for single-type infections. The protective effect of male circumcision was consistently observed in glans specimens, but not in shaft specimens.

The results of our study are remarkably similar to those of the two other RCTs of male circumcision previously reported.[11,22] Comparing point-prevalence in intention-to-treat analyses, our results for high-risk HPV comparing men in the circumcision to the control group at 24 months (PRR, 0.46; 95% CI, 0.38-0.57 for glans/sulcus specimens) are not different from the PRR of 0.66 (95% CI,

0.51-0.86) observed using urethral sampling at 21-months in Orange Farm, South Africa,[11] nor to the unadjusted risk ratio (RR) of 0.65 observed using glans/sulcus specimens at 24 months in Rakai.[21] Estimates of HPV incidence or clearance are not available for Orange Farm.[11] The RR of 0.67 (95% CI; 0.51-0.89) found in Rakai[14] for male circumcision on high-risk HPV incidence in intention-to-treat analyses is somewhat lower than our observed HR of 0.48 (95% CI, 0.40, 0.57) in glans specimens. This modest difference may largely be driven by the higher incidence rate among our Kenyan control group (55.0 per 100 person-years) as compared to controls in Rakai (29.4 per 100 person-years), who were older and more likely to be married, and thus a lower risk population than the younger, mostly unmarried male participants from Kisumu.

Male circumcision had a protective effect on the incidence of multiple (HR, 0.46) and single-type (HR, 0.77) infections, in contrast to Rakai, which found a protective effect on incident high-risk multiple infections (RR glans, 0.45), but not on single-type infections (RR, 0.89; 95% CI 0.60, 1.30) in intention-to-treat analyses.[14] Findings from Kisumu and Rakai showed increased high-risk HPV clearance in the circumcision group compared with the control group (HR Kisumu, 1.76; RR Rakai, 1.39).[14] Both the Kisumu and Rakai results appear to differ somewhat from the observational HIM study of 4,033 men,[23] which found that overall HPV incidence and persistence did not differ between circumcised and uncircumcised men; however, there were specific HPV types for which HPV incidence was lower, and clearance higher, in circumcised as compared to uncircumcised men, which is similar to our results. In the observational HIM study, associations between male circumcision and HPV incidence and clearance remained similar when the authors adjusted for sexual behavior of the participants. In our study, the effect of male circumcision on HPV incidence, clearance, and reinfection is also unlikely to be explained by changes in sexual behavior over time, as there was little difference in this aspect between the circumcision and the control group.[9]

This study is unique in that it examined the effect of male circumcision separately for glans/coronal sulcus and penile shaft specimens over time in an RCT setting. Until now it has been unclear whether HPV incidence differs by anatomical site.[15,22,24,25] We found a strong protective effect of male circumcision on incident HPV glans infections over 24 months (HR, 0.51), but not on shaft infections (HR, 1.01; 95% CI 0.87, 1.17). This is biologically plausible, since the inner foreskin is less keratinized than the shaft and, therefore, potentially more susceptible to HPV infection.[26] Furthermore, the foreskin likely creates a micro-environment that facilitates the persistence of penile HPV infection.[26] Accordingly, we found more frequent clearance of HPV in the circumcision group and of reductions in HPV prevalence over the 24 months of follow-up. In light of our findings of a lack of an association between male circumcision on the incidence and clearance of HPV infections of the penile shaft, further understanding is needed of the differential transmissibility of penile HPV infections of the shaft as compared to the glans/coronal sulcus, including modelling of the likely effect of male circumcision on the transmission of HPV from men to women based on these study findings. Data from the Rakai RCT showed a protective effect of male circumcision on HPV transmission from participating men to their female partners among HIV-negative couples.[27,28]

As study strengths, we utilized a sensitive and validated GP5+/6+ assay ascertained 44 HPV types and allowed determination of the clearance of any HPV, including high- and low-risk HPV within an RCT. Furthermore, we present novel data on observed associations between male circumcision and the occurrence of HPV reinfections, as well as clearance of newly acquired HPV infections. Our study also has some limitations: In our results presentation, we have utilized the term ‘re-infection” to refer to those type-specific infection groups which were observed following baseline positivity and subsequent negativity; however, these also could represent reactivation of latent viral infections.[29] β-globin positivity overall in glans and/or shaft samples ranged from 60-80% over study follow-up. However, we

observed similar results when analyses were restricted to β-globin positive samples. The observed relatively low prevalence of β-globin positivity at baseline and follow-up are not unexpected among penile HPV exfoliated cell samples. A possible explanation is that penile cells, particularly in shaft samples, are more keratinized and anucleated than those in the cervix, and therefore may contain relatively less human DNA.[18] A lower frequency of β-globin positivity in penile swab samples has also been documented in several studies of HPV in penile samples.[14] Furthermore, we were not able to examine the effect of male circumcision among HIV-positive men, or among men over 26 and under 18 years of age, given study eligibility criteria.

In 2007, the World Health Organization issued recommendations to promote male circumcision for HIV prevention.[30] Since then, over 27 million voluntary medical male circumcisions have been performed in 15 target countries in Eastern and Southern African.[31] Male circumcision may not be protective against *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, and *Trichomonas vaginalis* infections.[32] However, male circumcision is a valuable tool for HIV prevention,[9–11] and can also reduce the risk of HPV incidence, re-infection and increase HPV clearance. Given our results, male circumcision should be considered effective for preventing HPV infections and may thus synergistically with HPV vaccination programs contribute to the primary prevention of penile, anal, and cervical cancers.

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Author contributions:

J. S. Smith worked with R. C. Bailey, K. Agot, M. G. Hudgens, and C. Meijer to design the ancillary HPV study. C. Meijer conducted HPV testing of penile swab samples. H. Chakraborty, M.G. Hudgens, W. Mei, D. Backes and J. S. Smith collaborated on statistical analyses. J. S. Smith, E. Rohner and D. Backes participated in writing the manuscript. All authors reviewed and commented on the manuscript and approved its final submission.

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Table 1: Baseline characteristics

	Circumcision group	Control group	Overall
Demographic characteristics			
Age (years)╫	20 (19-22; 18-28; 1096)	20 (19-22; 17-24; 1097)	20 (19-22; 17-28; 2193)
Ethnic group			
Luo	1076 (98.2)	1085 (98.9)	2161 (98.5)
Other	20 (1.8)	12 (1.1)	32 (1.5)
Education level			
Less than secondary	372 (33.9)	391 (35.6)	763 (34.8)
Any secondary or above	724 (66.1)	706 (64.4)	1430 (65.2)
Employment status			
Employed and receiving a salary	94 (8.6)	98 (8.9)	192 (8.8)
Self-employed	303 (27.7)	294 (26.8)	597 (27.2)
Unemployed	699 (63.8)	705 (64.3)	1404 (64.0)
Occupation			
Professional/managerial	16 (1.5)	25 (2.3)	41 (1.9)
Skilled worker	108 (9.8)	87 (7.9)	195 (8.9)
Semi-skilled worker	73 (6.7)	74 (6.7)	147 (6.7)
Unskilled worker	565 (51.6)	606 (55.2)	1171 (53.4)
Farm laborer/fisherman	80 (7.3)	70 (6.4)	150 (6.8)
Student	254 (23.2)	235 (21.4)	489 (22.3)
Marital status			
Not married (no live-in partner)	1024 (93.8)	1018 (93.2)	2042 (93.5)
Not married (with live-in partner)	7 (0.6)	7 (0.6)	14 (0.6)
Married (not living with wife)	5 (0.5)	15 (1.4)	20 (0.9)
Married (living with wife)	56 (5.1)	52 (4.8)	108 (5.0)
Physical and laboratory findings			
Haemoglobin (g/L)	15.3 (14.2-16.3; 9.0-21.1; 1086)	15.3 (14.2-16.3; 8.3-20.1; 1085)	15.3 (14.2-16.3; 8.3-21.1; 2171)
Herpes simplex virus 2			
Positive	287 (27.3)	278 (26.5)	565 (26.9)
Negative	764 (72.7)	771 (73.5)	1535 (73.1)
Syphilis			
Positive	12 (1.1)	6 (0.6)	18 (0.9)
Negative	1043 (98.9)	1049 (99.4)	2092 (99.2)
Trichomonas vaginalis			

Positive	21 (1.9)	24 (2.2)	45 (2.1)
Negative	1064 (98.1)	1059 (97.8)	2123 (97.9)
Neisseria gonorrhoeae			
Positive	30 (2.8)	17 (1.6)	47 (2.2)
Negative	1053 (97.2)	1066 (98.4)	2119 (97.8)
Chlamydia trachomatis			
Positive	57 (5.3)	42 (3.9)	99 (4.6)
Negative	1025 (94.7)	1041 (96.1)	2066 (95.4)
Haemophilus ducreyi			
Positive	0 (0.0)	0 (0.0)	0 (0.0)
Negative	17 (100.0)	8 (100.0)	25 (100.0)
Sexual history with women			
Age at first sexual encounter (years)	16 (14-17; 5-22; 1056)	16 (14-17; 6-24; 1061)	16 (14-17; 5-24; 2117)
Sexual intercourse with any partner in previous 6 months			
Yes	956 (87.5)	957 (87.6)	1913 (87.5)
No	137 (12.5)	136 (12.4)	273 (12.5)
Number of partners in previous 6 months			
0	137 (12.5)	136 (12.4)	273 (12.5)
1	472 (43.2)	482 (44.1)	954 (43.6)
2+	484 (44.3)	475 (43.5)	959 (43.9)
Number of partners over lifetime	4 (3-7; 1-120; 1004)	4 (3-7; 1-86; 1016)	4 (3-7; 1-120; 2020)
Gave gifts or money to a woman for sexual intercourse in previous 6 months			
Yes	152 (15.8)	180 (18.7)	332 (17.2)
No	809 (84.2)	784 (81.3)	1593 (82.8)
Drank alcohol at last time of having sexual intercourse			
Yes	117 (10.7)	124 (11.3)	241 (11.0)
No	978 (89.3)	969 (88.7)	1947 (89.0)
Used a condom at last time of having vaginal sexual intercourse			
Yes	555 (50.7)	525 (48.0)	1080 (49.4)
No	540 (49.3)	568 (52.0)	1108 (50.6)
Used a condom with sexual intercourse in previous 6 months			
Always	210 (21.9)	208 (21.7)	418 (21.8)

Inconsistent	511 (53.3)	500 (52.1)	1011 (52.7)
Never	238 (24.8)	251 (26.2)	489 (25.5)
Bathing frequency			
Less than daily	23 (2.1)	22 (2.0)	45 (2.1)
Daily	1063 (97.9)	1062 (98.0)	2125 (97.9)

Note: Sample sizes vary slightly from the number of randomized participants due to different data sources.
╪Data are median (IQR; range; n) for ordinal data, or n (%) for categorical data.

Table 2: Prevalence of HPV infection in the glans or the shaft over 24 months among 2,193 men participating in a randomized, controlled trial of male circumcision, stratified by treatment arm

	Circumcision group (N=1,096) n (%)	Control group (N=1,097) n (%)	PRR (95% CI)
Baseline Visit‡			
HPV DNA positive	552 (50.4)	545 (49.7)	1.01 (0.93, 1.10)ǂ
High-risk HPV positive*	371 (35.7)	385 (36.6)	0.98 (0.87, 1.09)
Low-risk HPV positive	125 (12.0)	115 (11.0)	1.10 (0.87, 1.40)
Single HPV infections	230 (21.0)	231 (21.1)	1.00 (0.85, 1.17)
Multiple HPV infections	322 (29.4)	314 (28.6)	1.03 (0.90, 1.17)
6-month visit			
HPV DNA positive	287 (29.8)	440 (45.3)	0.66 (0.58, 0.74)
High-risk HPV positive	174 (18.3)	290 (30.2)	0.61 (0.51, 0.71)
Low-risk HPV positive	105 (11.0)	139 (14.5)	0.76 (0.60, 0.97)
Single HPV infections	162 (16.8)	210 (21.6)	0.78 (0.65, 0.94)
Multiple HPV infections	125 (13.0)	230 (23.7)	0.55 (0.45, 0.67)
12-month visit			
HPV DNA positive	262 (26.6)	479 (48.4)	0.55 (0.49, 0.62)≠
High-risk HPV positive	151 (15.7)	314 (32.7)	0.48 (0.40, 0.57)
Low-risk HPV positive	89 (9.2)	136 (14.2)	0.65 (0.51, 0.84)
Single HPV infections	173 (17.5)	226 (22.9)	0.77 (0.64, 0.92)
Multiple HPV infections	89 (9.0)	253 (25.6)	0.35 (0.28, 0.44)
18-month visit			
HPV DNA positive	270 (28.4)	474 (49.1)	0.58 (0.51, 0.65)
High-risk HPV positive	184 (19.8)	308 (32.3)	0.61 (0.52, 0.72)
Low-risk HPV positive	65 (7.0)	155 (16.3)	0.43 (0.33, 0.57)
Single HPV infections	170 (17.9)	222 (23.0)	0.78 (0.65, 0.93)
Multiple HPV infections	100 (10.5)	252 (26.1)	0.40 (0.33, 0.50)
24-month visit			
HPV DNA positive	225 (23.7)	378 (41.0)	0.58 (0.50, 0.66)┤
High-risk HPV positive	139 (14.8)	244 (26.8)	0.55 (0.46, 0.67)
Low-risk HPV positive	75 (8.0)	124 (13.6)	0.59 (0.45, 0.77)
Single HPV infections	136 (14.3)	173 (18.8)	0.76 (0.62, 0.94)
Multiple HPV infections	89 (9.4)	205 (22.2)	0.42 (0.33, 0.53)

Note: n: number; %: percentage; PRR: prevalence risk ratio (circumcision vs. control arm): CI: confidence interval; HPV: human papillomavirus; HR: high-risk; LR: low-risk.

Missing follow-up HPV result in circumcision arm: 6-month (n= 132); 12-month (n=109); 18-month (n=145); 24-month (n=145)

Missing follow-up HPV result in uncircumcision arm: 6-month (n= 125); 12-month (n=107); 18-month (n=132); 24-month (n=175)

*Infections with multiple HPV types were considered high-risk if one or more high-risk HPV types were detected. All other multiple infections were considered low-risk types unless they included HPVX.

‡All men were uncircumcised at the baseline visit , ǂ0.99 (0.89 – 1.10) in analyses restricted to beta-globin positive samples, ≠0.58 (0.51 – 0.68) in analyses restricted to beta-globin positive samples,

┤0.57 (0.49 – 0.67) in analyses restricted to beta-globin positive samples

Table 3: Incidence and reinfection of human papillomavirus (HPV) infections in the glans or the shaft over 24 months: hazard ratios and 95% confidence intervals (CIs) for the effect of male circumcision

	Incident infections in men negative for specific HPV type at baseline					Reinfections in men positive for specific HPV type at baseline*				
	Circumcision Arm (N=1096)		Control Arm (N=1097)		Hazard ratios (95% CI)	Circumcision Arm (N=1096)		Control Arm (N=1097)		Hazard Ratio (95% CI)
	Incident infections n/N	Person -Years†	Incident infections n/N	Person -Years†		Incident infections n/N	Person -Years†	Incident infections n/N	Person -Years†	
Any HPV	252/544	501.2	344/552	455.4	0.61 (0.52, 0.72)‡	212/504	247.8	195/397	149.1	0.66 (0.54, 0.81)≠
High-risk HPV	223/669	634.8	330/666	600.3	0.58 (0.49, 0.69)	105/356	192.3	137/340	140.1	0.57 (0.44, 0.74)
Low-risk HPV	199/915	850.5	313/936	875.4	0.61 (0.51, 0.73)	22/123	70.2	30/108	54.9	0.58 (0.33, 1.02)
HPV16/18	137/949	918.7	241/961	900.8	0.53 (0.43, 0.66)	21/146	91.7	26/130	73.1	0.69 (0.38, 1.23)
HPV 16/18/6/11	186/892	850.4	294/919	853.6	0.60 (0.50, 0.72)	38/201	124.7	44/166	91.4	0.66 (0.43, 1.03)
Single	365/866	794.8	437/866	766.4	0.77 (0.67, 0.89)	72/225	124.1	98/224	120.4	0.67 (0.50, 0.91)
Multiple	159/774	740.6	308/738	718.3	0.46 (0.38, 0.55)	70/315	184.4	99/279	122.8	0.46 (0.34, 0.63)
High-risk										
HPV16	109/983	954.4	185/994	941.1	0.57 (0.45, 0.72)	12/112	69.7	17/98	55.1	0.62 (0.29, 1.33)
HPV56	69/1037	1001.3	122/1027	982.2	0.54 (0.40, 0.72)	8/57	35.6	12/67	35.3	0.76 (0.30, 1.91)
HPV52	33/1056	1017.1	64/1040	998.3	0.51 (0.33, 0.77)	1/40	25.6	6/56	35.5	0.23 (0.03, 1.94)
HPV66	52/1040	1008.7	91/1047	1000.0	0.56 (0.40, 0.78)	3/54	30.4	7/50	30.3	0.40 (0.10, 1.57)
HPV35	46/1038	1002.7	109/1062	1008.3	0.41 (0.29, 0.58)	3/58	32.7	4/35	22.4	0.48 (0.10, 2.18)
HPV31	31/1053	1024.3	57/1057	1012.6	0.53 (0.34, 0.82)	1/43	26.8	1/39	20.7	---
HPV18	56/1049	1016.5	94/1060	1017.4	0.58 (0.42, 0.81)	2/47	30.7	4/37	22.0	0.33 (0.06, 1.87)
Low-risk										
HPV67	54/1048	1020.7	99/1034	990.8	0.53 (0.38, 0.74)	1/48	31.0	11/60	30.7	0.10 (0.01, 0.78)
HPV42	39/1045	1013.4	102/1044	993.7	0.37 (0.26, 0.53)	4/51	29.7	4/51	28.2	0.95 (0.23, 3.85)
HPVJC9710	47/1047	1001.9	119/1050	1003.3	0.38 (0.27, 0.54)	7/49	29.7	5/43	24.2	1.14 (0.36, 3.64)
HPV6	57/1048	1011.3	98/1059	1012.9	0.57 (0.41, 0.79)	4/48	29.8	5/36	22.0	0.59 (0.16, 2.24)
HPV40	41/1047	1019.4	76/1055	1013.1	0.53 (0.37, 0.78)	6/49	31.1	8/42	18.9	0.57 (0.19, 1.74)
HPV43	43/1048	1010.1	74/1054	1002.6	0.57 (0.39, 0.83)	5/48	26.1	6/42	19.0	0.65 (0.19, 2.16)
HPV11	36/1074	1036.2	51/1076	1028.1	0.70 (0.45, 1.07)	0/22	14.9	2/21	13.4	---

Note. n= number of men with a type-specific incident HPV infection; N= total number of men at risk for an incident infection of the specific HPV type.

*Analyses among men positive for the specific HPV type at baseline, then negative for that type during follow up. Only newly acquired (repeat infections) of the specific HPV type were considered incident infections.
† Person-years were estimated by assuming that the incident HPV infection was acquired at the midpoint between the last HPV-negative result and the first subsequent HPV-positive result.
‡0.56 (0.45, 0.70) in analyses restricted to beta-globin positive samples, ≠0.66 (0.50, 0.86) in analyses restricted to beta-globin positive samples
--- CI width > 1,000

Table 4: Clearance of human papillomavirus (HPV) infections in the glans or the shaft over 24 months: hazard ratios and 95% confidence intervals (CIs) for the effect of male circumcision

	Prevalent Infection present at baseline					Incident Infection not present at baseline				
	Circumcision Arm (N=1096 men)		Control Arm (N=1097 men)		Hazard ratios (95% CI)	Circumcision Arm (N=1096 men)		Control Arm (N=1097 men)		Hazard Ratio (95% CI)
	Cleared infections n/N	Person -Years*	Cleared infections n/N	Person -Years*		Cleared infections n/N	Person -Years*	Cleared infections n/N	Person -Years*	
Any HPV	1149/1160	421.8	1132/1171	533.6	1.87 (1.49, 2.34)ǂ	814/1194	306.5	1508/2333	703.6	1.68 (1.39, 2.02)≠
High-risk HPV	598/606	222.9	613/633	280.5	1.76 (1.29, 2.39)	431/627	156.8	735/1143	321.5	1.62 (1.25, 2.10)
Low-risk HPV	551/554	198.9	521/540	253.1	1.56 (1.21, 2.00)	383/567	149.7	773/1190	382.1	1.75 (1.33, 2.31)
HPV16/18	159/160	55.8	133/140	67.0	1.79 (1.29, 2.50)	109/165	41.6	181/278	78.1	---
HPV 16/18/6/11	229/230	83.3	189/198	88.8	1.53 (1.17, 1.99)	170/258	65.1	276/427	124.5	1.48 (0.99, 2.21)
Single	179/180	69.4	184/193	90.4	1.50 (1.14, 1.98)	423/606	161.2	763/1133	338.2	1.50 (1.17, 1.92)
Multiple	970/980	352.3	950/980	443.2	1.89 (1.46, 2.47)	364/554	148.8	788/1277	384.7	1.48 (1.13, 1.93)
High-risk										
HPV16	112/113	39.9	96/103	48.7	1.35 (0.87-2.08)├	73/109	28.2	118/184	51.3	1.31 (0.88, 1.95)
HPV56	57/59	25.0	66/70	36.5	1.23 (0.77, 1.94)	50/69	17.7	76/122	40.5	1.48 (0.90, 2.42)
HPV52	40/40	14.4	56/57	23.5	1.23 (0.66, 2.31)	23/32	8.2	36/64	11.2	---
HPV66	54/56	21.4	50/50	19.6	1.29 (0.67, 2.49)	31/52	15.8	58/91	28.6	1.11 (0.64, 1.94)
HPV35	58/58	24.3	35/35	12.9	0.94 (0.48, 1.82)	30/46	12.2	68/108	37.8	2.22 (1.23, 4.00)
HPV31	43/43	14.1	39/40	18.2	---	19/31	6.0	31/57	15.6	1.66 (0.71, 3.86)
HPV18	47/47	15.9	37/37	18.3	1.89 (1.02, 3.51)	36/56	13.4	63/94	26.7	1.40 (0.83, 2.37)
Low-risk										
HPV67	48/48	15.2	60/63	34.5	1.76 (0.99, 3.13)	29/54	10.2	55/99	38.9	1.95 (1.07, 3.54)
HPV42	51/51	18.5	51/53	25.2	1.25 (0.71, 2.20)	27/38	10.2	63/102	39.8	1.84 (1.01, 3.36)
HPVJC9710	49/49	16.8	43/47	20.6	1.28 (0.67, 2.46)	39/47	12.3	72/119	38.6	1.44 (0.87, 2.39)
HPV6	48/48	20.3	36/38	14.5	0.65 (0.32, 1.29)	40/57	14.3	59/98	31.9	0.95 (0.59, 1.54)
HPV40	49/49	15.2	41/42	22.2	---	25/41	9.7	47/76	25.8	1.27 (0.71, 2.26)
HPV43	48/48	21.5	42/43	26.1	1.25 (0.76, 2.06)	33/43	11.9	47/74	26.4	1.35 (0.74, 2.43)
HPV11	22/22	7.2	21/21	7.3	---	21/36	9.3	36/51	14.5	1.64 (0.76, 3.54)

Note. Clearance was defined as an HPV-positive result followed by an HPV-negative result for that type; n= number of cleared HPV infections; N=total number of HPV infections

* Person-years were estimated by assuming that an HPV infection was cleared at the midpoint between the last HPV-positive result and the first subsequent HPV-negative result.
ǂ 1.98 (1.48 – 2.66) in analyses restricted to beta-globin positive samples; ≠1.63 (1.26 – 2.10) in analyses restricted to beta-globin positive samples; ├1.18 (0.69 – 2.01) in Analyses restricted to beta-globin positive samples.
--- CI width > 1,000

Table 5: Prevalence of HPV infection over 24 months among in 2,193 men participating in a randomized, controlled trial of male circumcision, stratified by treatment arm and anatomical site

	Glans			Shaft		
	Circumcision Arm* (N=1,096) n (%)	**Control Arm† (N=1,097) n (%)**	**PRR (95% CI)**	**Circumcision Arm* (N=1,096) n (%)**	**Control Arm† (N=1,097) n (%)**	**PRR (95% CI)**
Baseline Visit‡						
HPV DNA positive	495 (45.16)	502 (45.76)	0.99 (0.90, 1.08)≠	209 (19.07)	189 (17.23)	1.11 (0.93, 1.32)├
High-risk HPV positive	316 (30.21)	351 (33.02)	0.92 (0.81, 1.04)	129 (12.02)	125 (11.65)	1.03 (0.82, 1.30)
Low-risk HPV positive	129 (12.33)	117 (11.01)	1.12 (0.89, 1.42)	57 (5.31)	40 (3.73)	1.43 (0.96, 2.12)
Single HPV infections	229 (20.89)	222 (20.24)	1.03 (0.88, 1.22)	128 (11.68)	118 (10.76)	1.09 (0.86, 1.37)
Multiple HPV infections	266 (24.27)	280 (25.52)	0.95 (0.82, 1.10)	81 (7.39)	71 (6.47)	1.14 (0.84, 1.55)
6-month visit						
HPV DNA positive	236 (24.56)	409 (42.08)	0.58 (0.51, 0.67)	154 (15.98)	128 (13.17)	1.21 (0.98, 1.51)
High-risk HPV positive	140 (14.63)	263 (27.25)	0.54 (0.45, 0.65)	91 (9.50)	80 (8.28)	1.15 (0.86, 1.53)
Low-risk HPV positive	92 (9.61)	139 (14.40)	0.67 (0.52, 0.86)	57 (5.95)	42 (4.35)	1.37 (0.93, 2.02)
Single HPV infections	143 (14.88)	200 (20.58)	0.72 (0.60, 0.88)	99 (10.27)	84 (8.64)	1.19 (0.90, 1.57)
Multiple HPV infections	93 (9.68)	209 (21.50)	0.45 (0.36, 0.57)	55 (5.71)	44 (4.53)	1.26 (0.86, 1.85)
12-month visit						
HPV DNA positive	211 (21.40)	431 (43.54)	0.49 (0.43, 0.56)	139 (14.08)	159 (16.06)	0.88 (0.71, 1.08)
High-risk HPV positive	123 (12.64)	279 (28.79)	0.44 (0.36, 0.53)	77 (7.91)	88 (9.02)	0.88 (0.65, 1.18)
Low-risk HPV positive	75 (7.71)	131 (13.52)	0.57 (0.44, 0.75)	49 (5.03)	57 (5.84)	0.86 (0.59, 1.25)
Single HPV infections	143 (14.50)	213 (21.52)	0.67 (0.56, 0.82)	106 (10.74)	107 (10.81)	0.99 (0.77, 1.28)
Multiple HPV infections	68 (6.90)	218 (22.02)	0.31 (0.24, 0.41)	33 (3.34)	52 (5.25)	0.64 (0.42, 0.98)
18-month visit						
HPV DNA positive	191 (20.13)	430 (44.61)	0.45 (0.39, 0.52)	153 (16.17)	175 (18.13)	0.89 (0.73, 1.09)
High-risk HPV positive	121 (12.98)	263 (27.63)	0.47 (0.39, 0.57)	105 (11.15)	110 (11.40)	0.98 (0.76, 1.26)
Low-risk HPV positive	53 (5.69)	155 (16.28)	0.35 (0.26, 0.47)	44 (4.67)	65 (6.74)	0.69 (0.48, 1.01)
Single HPV infections	126 (13.28)	216 (22.14)	0.59 (0.49, 0.72)	110 (11.63)	117 (12.12)	0.96 (0.75, 1.22)
Multiple HPV infections	65 (6.85)	214 (22.20)	0.31 (0.24, 0.40)	43 (4.55)	58 (6.01)	0.76 (0.52, 1.11)

24-month visit						
HPV DNA positive	175 (18.40)	356 (38.70)	0.48 (0.41, 0.56)	137 (14.42)	127 (13.77)	1.05 (0.84, 1.31)
High-risk HPV positive	107 (11.31)	222 (24.37)	0.46 (0.38, 0.57)	86 (9.13)	70 (7.63)	1.20 (0.88, 1.62)
Low-risk HPV positive	63 (6.66)	125 (13.72)	0.49 (0.36, 0.65)	43 (4.56)	52 (5.67)	0.81 (0.54, 1.19)
Single HPV infections	108 (11.36)	173 (18.80)	0.60 (0.48, 0.75)	87 (9.16)	78 (8.46)	1.08 (0.81, 1.45)
Multiple HPV infections	67 (7.05)	183 (19.89)	0.35 (0.27, 0.46)	50 (5.26)	49 (5.31)	0.99 (0.67, 1.45)

Note: n: number; %: percentage; PRR: prevalence risk ratio (circumcision vs. control arm): CI: confidence interval; HPV: human papillomavirus; HR: high-risk; LR: low-risk; Infections with multiple HPV types were considered high-risk if one or more high-risk HPV types were detected. All other multiple infections were considered low-risk types unless they included HPVX.

*Missing follow-up HPV result in circumcision arm: 6-month (n= 132); 12-month (n=109); 18-month (n=145); 24-month (n=145)

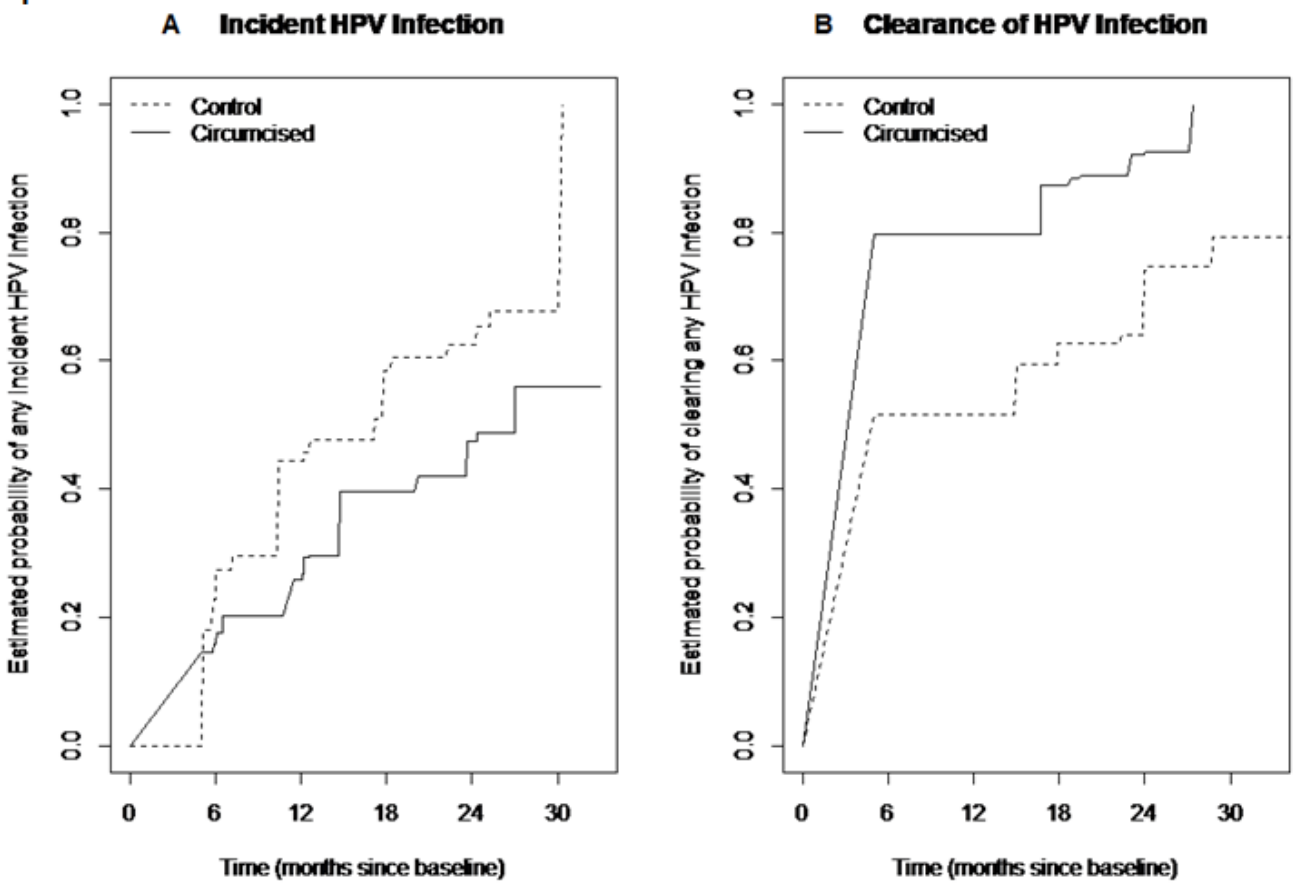
†Missing follow-up HPV result in uncircumcision arm: 6-month (n= 125); 12-month (n=107); 18-month (n=132); 24-month (n=175)

‡All men were uncircumcised at the baseline visit

≠0.95 (0.85 –1.07) in analyses restricted to beta-globin positive samples; ├1.08 (0.84 – 1.40) in analyses restricted to beta-globin positive samples.

Figure 1: Kaplan Meier curve of the cumulative incidence (A) and cumulative clearance (B) of penile HPV infections in the glans or shaft specimen, stratified by randomization arm in intention-to-treat analyses

1



Supplementary Table 1: Incident human papillomavirus (HPV) infections over 24 months among men negative for the given HPV type at baseline: hazard ratios and 95% confidence intervals (CIs) for the effect of male circumcision, stratified by treatment arm and anatomical site

	Glans					Shaft				
	Circumcision Arm* (N=1096)		Control Arm* (N=1097)		Hazard ratios (95% CI)	Circumcision Arm* (N=1096)		Control Arm* (N=1097)		Hazard Ratio (95% CI)
	Incident infections n/N	Person -Years†	Incident infections n/N	Person -Years†		Incident infections n/N	Person -Years†	Incident infections n/N	Person -Years†	
Any HPV	216/601	566.9	337/595	497.2	0.51 (0.43, 0.61)	333/887	827.8	338/908	847.0	1.01 (0.87, 1.17)
High-risk HPV	182/730	699.6	319/712	650.9	0.48 (0.40, 0.57)	236/944	902.9	240/947	897.8	0.98 (0.82, 1.17)
Low-risk HPV	173/917	858.2	308/946	882.8	0.54 (0.44, 0.65)	143/1016	969.8	168/1032	996.3	0.86 (0.68, 1.07)
HPV16/18	100/979	946.5	210/986	931.9	0.45 (0.35, 0.57)	88/1036	1005.2	118/1052	1008.1	0.74 (0.56, 0.98)
HPV 16/18/6/11	136/927	886.8	262/947	887.1	0.49 (0.40, 0.60)	136/1021	982.3	161/1039	995.7	0.85 (0.68, 1.07)
Multiple	133/830	801.9	295/817	752.9	0.39 (0.32, 0.47)	133/1015	976.4	153/1026	982.3	0.87 (0.69, 1.10)
High-risk										
HPV16	71/1011	980.1	156/1015	963.9	0.44 (0.33, 0.58)	71/1047	1014.8	88/1064	1021.2	0.82 (0.60, 1.12)
HPV56	56/1046	1013.5	105/1035	988.6	0.51 (0.37, 0.70)	41/1078	1045.0	53/1073	1035.1	0.76 (0.50, 1.14)
HPV52	28/1060	1022.6	59/1046	1006.4	0.47 (0.30, 0.73)	16/1091	1055.6	16/1089	1043.9	0.99 (0.49, 1.98)
HPV66	46/1048	1019.6	78/1053	1003.8	0.57 (0.40, 0.83)	35/1079	1043.6	38/1083	1041.0	0.92 (0.58, 1.45)
HPV35	41/1042	1007.3	97/1064	1010.4	0.42 (0.29, 0.60)	35/1083	1045.8	33/1089	1043.1	1.06 (0.66, 1.70)
HPV31	22/1065	1034.6	48/1064	1020.7	0.45 (0.27, 0.74)	24/1076	1043.1	20/1088	1043.8	1.20 (0.66, 2.18)
HPV18	46/1057	1024.6	84/1066	1024.6	0.54 (0.37, 0.77)	27/1083	1049.4	40/1083	1041.6	0.66 (0.41, 1.08)
Low-risk										
HPV67	42/1054	1023.8	90/1041	995.4	0.45 (0.31, 0.66)	35/1085	1055.1	50/1081	1044.0	0.70 (0.45, 1.08)
HPV42	30/1052	1019.9	95/1049	1001.2	0.30 (0.20, 0.46)	29/1079	1045.1	26/1084	1042.6	1.11 (0.65, 1.89)
HPVJC9710	32/1053	1009.6	106/1053	1005.5	0.29 (0.20, 0.43)	30/1083	1044.5	52/1080	1041.0	0.57 (0.36, 0.89)
HPV06	41/1054	1014.7	86/1062	1015.7	0.47 (0.32, 0.68)	38/1085	1051.2	44/1087	1045.1	0.86 (0.55, 1.32)
HPV40	35/1052	1023.3	71/1060	1019.6	0.49 (0.43, 0.73)	22/1084	1055.1	22/1089	1045.7	0.99 (0.55, 1.79)

HPV43	33/1056	1016.8	67/1056	1007.8	0.48 (0.32, 0.73)	30/1080	1041.6	36/1087	1041.2	0.83 (0.51, 1.35)
HPV11	21/1075	1039.9	40/1080	1030.8	0.52 (0.31, 0.88)	24/1089	1049.6	25/1091	1048.7	0.96 (0.55, 1.67)

Note. n= number of men with a type-specific incident HPV infection; N= total number of men at risk for an incident infection of the specific HPV type

*Analyses among men negative for specific HPV type at baseline

† Person-years were estimated by assuming the incident HPV infection was acquired at the midpoint between the last HPV-negative result and the first subsequent HPV-positive result

Supplementary Table 2: Clearance of human papillomavirus (HPV) infections present at baseline over 24 months: hazard ratios and 95% confidence

intervals (CIs) for the effect of male circumcision on HPV clearance, stratified by treatment arm and anatomical site

	Glans					Shaft				
	Circumcision Arm* (N=1096 men)		Control Arm* (N=1097 men)		Hazard ratios (95% CI)	Circumcision Arm* (N=1096 men)		Control Arm* (N=1097men)		Hazard Ratio (95% CI)
	Cleared infections n/N	Person -Years†	Cleared infections n/N	Person -Years†		Cleared infections n/N	Person -Years†	Cleared infections n/N	Person -Years†	
Any HPV	984/992	363.1	1010/1040	475.5	1.90 (1.49, 2.42)	321/323	102.0	297/301	107.0	2.19 (1.34, 3.58)
High-risk HPV	499/504	188.1	532/547	246.1	1.73 (1.27, 2.37)	182/184	59.1	171/172	58.8	1.15 (0.59, 2.25)
Low-risk HPV	485/488	175.0	478/493	222.4	1.54 (1.29, 1.85)	139/139	42.8	126/129	48.3	1.31 (0.79, 2.18)
HPV16/18	124/124	43.0	108/113	55.4	1.83 (1.26, 2.65)	61/62	20.6	47/47	15.3	---
HPV 16/18/6/11	187/187	68.3	158/164	74.8	1.49 (1.12, 1.98)	79/80	27.6	62/63	21.3	1.01 (0.58, 1.77)
Multiple	803/810	293.3	826/850	384.9	1.96 (1.47, 2.61)	215/217	65.6	205/206	75.2	0.88 (0.53, 1.48)
High-risk										
HPV16	85/85	29.5	77/82	39.0	1.28 (0.80, 2.06)	48/49	16.8	33/33	11.1	---
HPV56	49/50	22.2	59/62	33.1	1.28 (0.80, 2.06)	18/18	5.2	24/24	8.3	---
HPV52	36/36	13.6	51/51	20.4	1.36 (0.72, 2.56)	5/5	1.6	8/8	3.2	---
HPV66	47/48	18.1	44/44	17.5	1.31 (0.67, 2.58)	16/17	5.8	14/14	4.6	---
HPV35	54/54	22.0	33/33	11.6	---	13/13	4.2	8/8	3.1	---
HPV31	31/31	10.4	32/33	16.0	---	20/20	5.8	9/9	2.5	---
HPV18	39/39	13.5	31/31	16.5	1.98 (1.02, 3.82)	13/13	3.9	14/14	4.2	---
Low-risk										
HPV67	42/42	13.6	53/56	31.0	1.77 (0.97, 3.22)	11/11	3.0	16/16	6.7	---
HPV42	44/44	16.5	46/48	23.9	1.26 (0.69, 2.27)	17/17	4.9	13/13	4.3	---
HPVJC9710	43/43	15.0	42/44	18.4	1.43 (0.75, 2.71)	13/13	4.1	15/17	5.7	---
HPV06	42/42	18.4	33/34	13.3	0.62 (0.30, 1.26)	11/11	4.3	9/10	4.5	---

HPV40	44/44	13.9	36/37	17.8	---	12/12	3.3	8/8	4.2	---
HPV43	40/40	15.1	40/41	25.3	2.02 (1.06, 3.83)	16/16	5.1	10/10	3.7	---
HPV11	21/21	6.9	17/17	6.1	---	7/7	2.6	6/6	1.5	---

Note. Clearance was defined as an HPV-positive result followed by an HPV-negative result; n= number of cleared HPV infections; N=total number of HPV infections present at baseline

*Analyses among infections present at baseline

† Person-years were estimated by assuming an HPV infection was cleared at the midpoint between the last HPV-positive result and the first subsequent HPV-negative result.

--- CI width > 1,000

Supplementary Table 3: Reinfections of human papillomavirus (HPV) over 24 months among men positive for the given HPV type at baseline, then negative for that type during follow-up: hazard ratios and 95% confidence intervals (CIs) for the effect of male circumcision stratified by treatment arm and anatomical site

	Glans					Shaft				
	Circumcision Arm* (N=1096)		Control Arm* (N=1097)		Hazard ratios (95% CI)	Circumcision Arm* (N=1096)		Control Arm* (N=1097)		Hazard Ratio (95% CI)
	Incident infections n/N	Person -Years†	Incident infections n/N	Person -Years†		Incident infections n/N	Person -Years†	Incident infections n/N	Person -Years†	
Any HPV	157/464	233.6	186/387	147.2	0.52 (0.42, 0.65)	53/199	119.0	61/180	102.8	0.71 (0.49, 1.03)
High-risk HPV	73/306	166.4	117/318	136.9	0.53 (0.39, 0.72)	21/126	79.4	28/122	72.8	0.68 (0.38, 1.20)
Low-risk HPV	20/129	73.8	33/112	54.3	0.44 (0.25, 0.78)	8/57	36.4	7/37	21.6	0.65 (0.23, 1.81)
HPV16/18	16/117	74.0	17/107	61.5	0.88 (0.43, 1.79)	3/59	36.6	2/45	27.0	1.10 (0.18, 6.70)
HPV 16/18/6/11	24/167	102.8	34/142	79.2	0.57 (0.34, 0.98)	6/74	46.9	7/57	35.5	0.64 (0.21, 1.92)
Multiple	48/256	150.3	88/257	119.1	0.41 (0.29, 0.59)	9/80	52.1	14/71	40.8	0.50 (0.21, 1.14)
HPV16	8/85	52.8	10/79	44.7	0.86 (0.32, 2.31)	2/48	30.3	0/33	19.0	---
HPV56	7/49	29.8	13/60	30.1	0.63 (0.25, 1.62)	0/18	11.6	3/24	14.6	---
HPV18	2/39	25.4	2/31	17.9	0.69 (0.09, 5.1)	0/13	8.7	1/14	8.7	---

Note. n= number of men with a type-specific incident HPV infection; N= total number of men at risk for an incident infection of the specific HPV type.
*Analyses among men positive for the specific HPV type at baseline, then negative for that type during follow up. Only new repeat infections of the specific HPV type were considered incident infections.
† Person-years were estimated by assuming the incident HPV infection was acquired at the midpoint between the last HPV-negative result and the first subsequent HPV-positive result.
--- CI width > 1,000

Supplementary Table 4: Clearance of incident human papillomavirus (HPV) infections not present at baseline over 24 months: hazard ratios and 95% confidence intervals (CIs) for the effect of male circumcision on HPV clearance, stratified by treatment arm and anatomical site

	Glans					Shaft				
	Circumcision Arm* (N=1096 men)		Control Arm* (N=1097 men)		Hazard ratios (95% CI)	Circumcision Arm* (N=1096 men)		Control Arm* (N=1097 men)		Hazard Ratio (95% CI)
	Cleared infections n/N	Person -Years†	Cleared infections n/N	Person -Years†		Cleared infections n/N	Person -Years†	Cleared infections n/N	Person -Years†	
Any HPV	606/885	224.4	1315/2083	621.5	1.76 (1.43, 2.17)	499/751	174.2	582/836	178.8	0.68 (0.47, 0.98)
High-risk HPV	316/463	112.0	662/997	278.5	1.84 (1.34, 2.53)	264/396	91.7	296/421	88.4	0.52 (0.30, 0.89)
Low-risk HPV	290/422	112.4	693/1086	343.0	1.78 (1.29, 2.46)	286/415	90.4	235/355	82.4	0.73 (0.41, 1.31)
HPV16/18	77/117	29.4	153/239	66.5	1.58 (0.67, 3.76)	66/98	23.5	90/128	27.7	0.54 (0.15, 1.98)
HPV 16/18/6/11	118/179	45.9	234/365	107.4	1.46 (0.88, 2.42)	107/160	36.4	134/197	41.5	0.72 (0.34, 1.50)
Multiple	269/432	113.8	714/1178	349.6	1.33 (0.98, 1.79)	299/462	112.2	367/551	115.8	0.60 (0.36, 1.00)
HPV16	47/71	17.6	97/155	42.8	1.91 (1.06, 3.43)	49/71	18.2	64/88	19.6	0.76 (0.40, 1.44)
HPV56	37/56	12.8	64/105	35.0	2.04 (1.07, 3.88)	27/41	8.9	35/53	11.3	0.94 (0.42, 2.13)
HPV18	30/46	11.8	56/84	23.7	1.42 (0.79, 2.56)	17/27	5.3	26/40	8.1	---

Note. Clearance was defined as an HPV-positive result followed by an HPV-negative result; n= number of cleared HPV infections; N=total number of HPV infections

*Analyses among HPV infections not present at baseline

† Person-years were estimated by assuming an HPV infection was cleared at the midpoint between the last HPV-positive result and the first subsequent HPV-negative result.

--- CI width > 1,000