HUMAN PAPILLOMAVIRUS TYPE COMPETITION AND THE ASSOCIATIONS BETWEEN HPV TYPES AND FUTURE HPV ACQUISITION IN MEN

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ABSTRACT

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Human papillomavirus type competition and the associations between HPV types and future HPV acquisition in men
(Under the direction of Jennifer S. Smith)

Infection with human papillomavirus (HPV) is the primary cause of cervical cancer and other anogenital cancers. Multiple HPV infections have been detected in up to 70% of HPV infected men, yet there are no data on the association between HPV types in men, which may impact the type-specific efficacy of HPV vaccination through HPV type competition and replacement.

In a cohort of uncircumcised, human immunodeficiency virus (HIV)-seronegative men aged 17–28 years from Kisumu, Kenya, we assessed the associations between HPV types at baseline (N=2,702) using semi-Bayesian multivariate logistic regression. In a prospective analysis (N=1,064) we used parametric survival models to compare rates of acquisition of HPV infections among men infected and uninfected with vaccine-relevant types HPV-16, 18, 31, 45, 6, and 11 at baseline.

Half of all men were HPV positive at baseline, of whom 57% had multiple HPV infections. In the cross-sectional analysis, HPV types 31, 39, 56, 58, and 59 were positively associated with both vaccine types HPV-16 and 18 (two-sided p-value <0.05); no negative associations between individual HPV types were observed.

Over 2,462 person-years of follow-up, 2,233 incident HPV infections were detected. Men with HPV-18, 31, 45, or 11, but not HPV-16 or 6, had higher rates of any-HPV and

high-risk (HR) HPV acquisition compared to men without each HPV type at baseline. Relative rates of acquisition of individual HR-HPV types varied by baseline HPV type; however, we did not observe a clear pattern of HPV acquisition by degree of phylogenetic relatedness to the baseline infection.

Except for HPV-39 acquisition among men with HPV-6 (aHR: 0.1 (0.0, 0.8)), there was no evidence of negative associations between HPV types or reduced HPV acquisition among men with baseline infections that indicate a strong potential for HPV type competition. To better understand the potential for changes in the HPV type distribution following HPV vaccination, future studies that monitor HPV type distributions in pre- and post-vaccinated populations are needed.

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aHR: adjusted hazard ratio

aMSR: adjusted median survival ratio

ASCUS: atypical squamous cells of undetermined significance

CI: confidence interval

DAG: directed acyclic graph

DNA: deoxyribonucleic acid

EIA: enzyme immunoassay

FDA: Food and Drug Administration

HIV: human immunodeficiency virus

HPV: human papillomavirus

HR: high-risk

HSV-2: herpes simplex type 2

LR: low-risk

LSIL: low-grade squamous intraepithelial lesions

MLE: maximum likelihood estimation

n: number

NPMLE: nonparametric maximum likelihood estimation

OR: odds ratio

p: statistical p-value

PCR: polymerase chain reaction

RCT: randomized control trial

RLBH: reverse line blot hybridization

STI: sexually transmitted infection

UNIM: University of Nairobi, Illinois and Manitoba

VIF: variance inflation factor

I: INTRODUCTION

Human papillomavirus infection

Oncogenic human papillomavirus (HPV) infection is one of the most prevalent sexually transmitted infections (STI) worldwide and is a necessary cause of invasive cervical cancer[1-3]. It is responsible for other anogenital cancers, including vaginal and vulvar cancer in women, and anal and penile carcinoma in men. There are over 130 types of HPV, 40 of which infect the anogenital tract of men and women. Fourteen types of HPV are considered oncogenic or high-risk (HR) HPV types because they are found in invasive cervical cancer biopsy specimens and laboratory data have shown their oncogenic potential. HR-HPV infection is considered a necessary cause of cervical precancer and invasive cervical cancer among women[1, 3, 4]. HR-HPV types include 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68. Types classified as low oncogenic risk (LR) HPV include 6, 11, 26, 30, 32, 34, 40, 42, 43, 44, 53, 54 55, 57, 61, 64, 67, 69, 70, 71 (equivalent to CP8061), 72, 73, 81 (equivalent to CP8304), 82 (IS39 and MM4 subtypes), 83 (equivalent to MM7), 84 (equivalent to MM8), 85 (cand85), 86, 89 (cand89 equivalent to CP6108), and JC9710.

There are currently two FDA-approved HPV vaccines that provide type-specific protection against HPV infection in both men and women. Merck produces a quadrivalent HPV vaccine that protects against high-risk HPV types 16 and 18, and against LR-HPV types 6 and 11[5-7]. Nearly 70% of cases of cervical cancer have been attributed to infection with HPV types 16 and 18 and types 6 and 11 are responsible for the majority (~90%) of genital warts[1, 8]. The bivalent vaccine produced by GlaxoSmithKline provides protection against HPV-16 and 18, and studies show cross-protection against HR-HPV types 31 and 45[9-11].

Penile HPV infection

The prevalence of HPV in men has been reported to be 10-75% in men, and can vary greatly depending on the characteristics of the study population, sample collection method (e.g. swab, emery paper for exfoliated cells), anatomical site sampled, and the sensitivity of the laboratory HPV assay employed[12-15]. HPV has been associated with 70-100% of penile intraepithelial neoplasia and 40-50% of invasive penile cancer[16-18]. Although the burden of HPV-associated penile lesions and cancer is relatively low compared to other cancers in men such as anal cancer[19], rates of penile cancer have been correlated to rates of cervical cancer at the population-level[20]. Men are important carriers of HPV infection and may transmit their HPV infection to their female partners susceptible to the development of cervical cancer[21, 22].

HPV deoxyribonucleic acid (DNA) has been detected in exfoliated cells from the penile shaft, glans, coronal sulcus, urethra, scrotum and anus, and in semen and urine[12, 15]. Relative to cervical HPV infection, our knowledge of penile HPV in men is limited. There are an overwhelming number of asymptomatic HPV infections in men with subclinical penile lesions, and there are no routine screening programs for HPV infection or HPV-associated genital lesions in men. However, studies suggest that the prevalence of male penile HPV infection is at least as high as cervical HPV prevalence in women[23-30]. Detection of penile HPV DNA has been associated with lack of circumcision[26, 28, 31], decreased condom use[31, 32], and increased frequency and number of sexual contacts[25, 27, 28, 31].

Multiple HPV infections and type competition

Coinfection with multiple HPV types has been observed in 20-73% of HPV infected males[14, 32-39] and have been associated with an increased risk of HPV acquisition of additional HPV types[35, 40-43], HPV persistence[33, 35, 40, 44, 45], and development of cervical precancerous lesions in women[46-48]. Multiple HPV infections could have an impact on the efficacy of the HPV vaccine due to type competition or type replacement. HPV type replacement, a change in the distribution of HPV types found in the population when specific HPV types are reduced or eliminated by vaccination, is an important concern related to mass HPV vaccination. Type competition may result from some yet unknown biological mechanism, whereby infection with one HPV type inhibits the acquisition or persistence of other HPV types[35, 42]. Thus, when one HPV type is prevented by vaccination, the prevalence of the other HPV type could potentially increase. In contrast, if infection with one HPV type facilitates the acquisition or persistence of other HPV types, it is possible that when one HPV type is prevented by vaccination, the other type might be reduced in the population following wide-spread vaccination.

If HPV types do compete, this will be reflected in the population as a low probability of coinfection with two specific HPV types. However, there are several reasons why, in addition to the possibility of type competition, that two HPV types may be unlikely to occur together in coinfection. The prevalence of HPV types detected in a sample population are dependent on the HPV type distribution found in the general population in that specific geographical area and the HPV types circulating within sexual contacts from whom they acquired their HPV infections. In addition, observed positive associations between HPV types may be due to the common transmission route and risk factors for all HPV types, such

as age[49-51], condom use[37], circumcision status[34, 52], and lifetime[37, 53, 54] or recent[50] number of sexual partners. Thus, associations between HPV types reflect a combination of exposure to HPV, the circulating genotype distribution, individual behavioral characteristics, host susceptibility (e.g. acquired immunity or cross-protection), or molecular interactions between HPV types that inhibit or facilitate infection with other HPV types[35, 42, 55].

Molecular studies of multiple HPV infections

Numerous studies have detected two or more HPV types in cervical and penile samples and these studies provide evidence that more than one HPV type can co-exist in the same tissue. However, only a few studies have examined molecular interactions between HPV types within individual cells[56, 57]. Simultaneous infection with HPV-40, HPV-11 and HPV-82 led to regionally separate HPV infections within a single human foreskin xenograft in mice[56]. These results suggest HPV types may interact in a way that results in the expansion of a dominate type and a concomitant exclusion of other types[56]. An earlier study found that several HR-HPV type combinations, electroporated into primary keratinocytes, could occupy and replicate within a single cell[57]. This study found evidence that HPV types may interact in coinfected cells to produce survival and replication advantages for some types, while hindering proliferation of other types. For example, HPV-18 maintained higher genomic copies when coinfected with HPV-31 and 39 than when singly infected. It is possible that one viral type can out-compete other types for the necessary replication factors, hindering survival and replication of certain types in coinfection[57].

Literature review

Currently, there is no direct evidence from prospective studies of vaccinated women that certain HPV types are either reduced or increased in the population as a result of vaccination. A limited number of studies in women provide information on whether specific HPV types are likely to coinfect the genitals[49, 51, 53, 58-63], risk factors for multiple HPV infections[50, 53, 63], and whether genital infection with one HPV type modifies the risk of acquisition or clearance of another HPV type[40-43, 51]. However, there are no studies of multiple HPV infections and type competition in African populations, where the HPV genotype distribution may differ from those in North America, Europe or South America, and there are no studies in men.

Previous cross-sectional studies in women have found that the prevalence of multiple infections and the number of HPV types detected are observed more often than would be predicted by chance alone (Table 1.1)[40-42, 58-60]. Studies have also reported several HPV genotypes and phylogenetic clades to be associated with concurrent infection with other types and clades. Among HPV-positive women, one study found HPV-52 and HPV-68 in combination with HPV-16, and HPV-18 in combination with HPV6/11 to occur less often than expected ($p<0.05$)[51]. A recent study from Denmark found 31 pair-wise type combinations to be positively associated ($p<0.05$) and found 49 negative associations among 351 HPV pair combinations ($p<0.05$)[60]. However, these women were referred for testing based on clinical suspicion of infection and only HPV-51 was consistently associated with HPV-16[60]. A pooled analysis of women worldwide (n=13,961) found positive associations, but no negative associations, between specific HPV pairs: HPV33+35, HPV33+58, HPV33+39, HPV18+45, HPV31+35 ($p<0.01$)[58]. HPV clade-A9 (HPV-16, 31,

33, 35, 52, 58) was found less often in multiple HPV infections as compared to all other HPV clades (OR: 0.68; 95% CI: 0.48, 0.95) in a population of HIV-infected and uninfected women from the United States[59].

Numerous prospective studies have found that women with HPV infection at baseline are more likely to acquire additional HPV types and that acquisition of multiple HPV types occurs more often than expected[40, 42, 43]. In addition, several papers have addressed associations between individual HPV types and HPV acquisition(Table 1.2). In analyses limited to five HPV DNA types, current infection with HPV-16, 18, 31, 45 or 6 did not predict future acquisition of any other HPV type among female University students[42]. On the other hand, in a population of cytologically normal women, there was an increased odds of subsequent HPV DNA detection of species A7 genotypes among women with HPV-16 DNA at baseline compared to women without HPV-16. In agreement with results from the ASCUS/LSIL Triage Study[55], any HPV infection, regardless of type, was associated with an increase in HPV acquisition of other types due to a common mode of transmission or common risk factors[43]. A pre-existing HPV-16 infection predisposed young women to acquiring another HPV type infection but did not affect the persistence of other type infections[43]. This finding is consistent with a study of low-income women from Brazil that reported an effect of existing infection on the acquisition of another type but not an effect on persistence[40]. In contrast, incident infections with HPV-16 and 18 were associated with a higher odds of acquiring HPV-58, but not acquiring other types, among cytologically normal women from Colombia[41].

Table 1.1. Summary of cross-sectional studies on the number of HPV infections and associations between HPV types

Reference	Population (sample size)	Association	Study results	Notes
Vaccarella (2010)	IARC pooled analysis of women worldwide (n=13,961)	Number of HPV types detected (0/E ratio and 95% CI)	1 infection=0.67 (0.65, 0.69)	-All results moved towards null in random effects models
			2 infections=1.62 (1.49, 1.74)	-Greater number of infections than expected only evident with EIA not line blot
			3+ infections=6.43 (5.31, 7.62)	
		Positive association between HPV pairs ($p<0.01$)	HPV33+35, HPV33+58, HPV33+39, HPV18+45, HPV31+35	-4 of 5 pairs that occurred together more often than expected were from the same alpha-species
		Negative association between HPV pairs ($p<0.01$)	HPV16+81	-From different alpha-species
Chaturvedi (2005)	HIV- (n=854) and HIV+ (n=275) American women	Negative association between clade-A9 and multiple infections	Clade-A9 vs. all other clades adjusted OR=0.68 (0.48, 0.95)	-No association between other clades and multiple infection
		Number of HPV types detected (0/E ratio and 95% CI)	1 infection=0.67 (0.59, 0.76)	
			2 infections=0.78 (0.64, 0.95)	
			3 infections=1.56 (1.15, 2.13)	
			4+ infections=6.91 (4.76, 9.71)	
Mejlhede (2010)	Danish women suspected of cervical HPV infection (n=3,588)	Number of HPV types detected (0/E ratio and 95% CI)	1 infection=0.60 (0.60, 0.60)	-Clear trend and significant results actually presented for 0-8+ infections
			3 infections=1.20 (1.10, 1.40)	
			5 infections=8.1 (5.80, 10.30)	
			7 infections=80.6 (10.0, 151.3)	

		Positive association between HPV pairs	31 type pairs positively associated at $p<0.05$, 16 of these significant at $p<0.01$	
		Negative association between HPV pairs	49 type pairs negatively associated at $p<0.05$, only 1 of these significant at $p<0.01$	
		Negative association between HPV pairs	Only HPV16 had OR<1.0 for all pair-wise combinations	-Not all had 95% CIs that excluded the null
Tota (2010 conference abstract)	Ludwig McGill (n=2,462), BCCR (n=1,500), HITCH (n=503), CCCaST (n=10,154)	Negative association between HPV pairs (0/E ratio with 95% CI)	All confidence intervals for O/E ratios that were <1.0 included the null	-Authors interpret their results as evidence against type replacement since no significant O/E ratios <1.0
Oliveira (2008)	Brazilian women with previous abnormal cytology (n=232)	Clade distribution	Clade-A10 occurred most frequently in multiple infections, despite clade-A9 representing the five most prevalent types	-Article focused on the association between multiple infections and cervical outcomes
Rousseau (2003)	Brazilian women enrolled in a prospective cohort (n=2,075)	Positive association between HPV16/HPV18 and other HPV types ($p<0.05$)	-Among all women: 7 with HPV16, 1 with HPV18 -HPV-positive women: none	-Prospective data on acquisition also presented
		Negative association between HPV pairs ($p<0.05$)	-Among all women: none -HPV-positive women: HPV16 with HPV52 and 58, HPV18 with HPV6/11	

Table 1.2. Summary of prospective studies on the associations between HPV types and subsequent acquisition

Reference	Population (sample size)	Association	Study results	Notes
Thomas (2000)	American female university students enrolled in a prospective cohort (n=518)	Concurrent acquisition of multiple HPV types	Num. visits where women concurrently acquired 2-3 types was greater than expected	-Concurrent acquisition occurred more than expected, however, no differences by HPV type
		Pair-wise associations between types acquired together	Associations between HPV-18, 31 and 6 were all positive and ranged from 5.8 to 20.7	
		Sequential acquisition of multiple HPV types	No differences between expected and observed acquisitions of five HPV types after infection with another type	-The risk of acquiring a new HPV type was not related to the previous infection type.
		Pair-wise associations between types acquired sequentially	No significant negative HR for predictive HPV types on the acquisition of other HPV types; HR for HPV-6 and 45=8.7 (1.3, 57.6)	
Rousseau (2001)	Brazilian women enrolled in a prospective cohort (n=1,860)	Association between baseline type-specific infections and any or HR acquisition	HPV-16 and 18 at baseline=greatest risk of acquisition; risk of acquisition of 16 not elevated among HPV+ vs. HPV- at baseline	-Women with baseline infections more likely to acquire any and HR-HPV, regardless of baseline type.
			No negative HR's for group of type-specific acquisition; most HR's strongly positive (1.9-8.1)	
Liaw (2001)	Cytologically normal women attending gynecology clinics in US (n=1,124)	Comparison of type-specific acquisition among HPV+ vs. HPV- at baseline	No negative ORs for acquisition of any type; ORs ranged from 4.6-12.0.	-Prevalent HPV-16 infection was generally, not-specifically, associated with an increased risk of subsequent infection

Rousseau (2003)	Brazilian women enrolled in a prospective cohort (n=2,075)	O/E for HPV-16 and 18 in combination with other specific HPV types	Greater than expected frequencies for most HPV pairs; when limited to just HPV-infected women, fewer HPV-16 and 52, 16 and 58, and 18 and 6/11 infection pairs than expected if independent	-Had analysis limited to HPV+ only women (e.g. minimize the effect of common risk factors)
Mendez (2005)	Cohort study in Colombia (n=1,857)	O/E number of HPV types per visit	Concurrent acquisition of multiple types occurred more than expected	-No evidence of type competition; study lacked power to detect many pair-wise associations for HPV acquisition.
		Pair-wise associations between baseline type and acquired HPV type	No negative ORs for type-specific acquisitions. Pair-wise associations ranged from 5.9-16.2. HPV-16 and 18 increased the odds of acquiring HPV-58	-Analyzed vaccine-type groups (e.g. HPV-16/18/6/11)--positive associations with specific HPV types.
Plummer (2007)	ASCUS/LSIL triage study (n=4,504)	"HPV interactions for incident infection" e.g. the effect of current type infection on subsequent acquisitions	Only significant association was HPV-16 and 31 (OR=1.95), all other non-significant OR's ranged from 0.57-1.52	-Included frailty term in models -Concluded no interaction between multiple HPV types and acquisition--infections are fundamentally independent of each other.

Conclusions

When carcinogenic HPV infections are prevented by mass vaccination, there is a theoretical possibility that this could result in other HR-HPV types filling the ecological niche of HPV-16 and 18[64]. However, there is no direct evidence from prospective studies of vaccinated individuals that non-vaccine HPV types are either reduced or increased as a result of vaccination against HPV. Until there are large populations of vaccinated individuals, the research community has responded to this potential concern by examining the associations between HPV types in observational studies of women. Since there are reported differences in genotype distribution across gender and geographical regions[12, 14, 65], the potential for HPV type replacement could differ across study populations. Given the recent approval of HPV prophylactic vaccination for young men[66], data on HPV coinfections in men are needed to assess associations between HPV types and the potential for future HPV type replacement in men.

II: SPECIFIC AIMS

Currently, there are no studies on HPV type competition and associations between HPV types throughout the natural history of HPV infections in men. Therefore, using data from a randomized control trial (RCT) of male circumcision in Kisumu, Kenya, we aim:

Specific Aim #1. To determine the type-specific associations between vaccine preventable HPV types and all other HPV types.

Aim 1.1. To determine the correlates of multiple HPV type infections.

Aim 1.2. To determine whether multiple HPV infections occur more often than expected under the assumption of independence.

Aim 1.3. To determine the association between each of the four vaccine-preventable HPV types and all other HPV types.

Hypothesis. We hypothesize that multiple infections will occur more than expected under the assumption of independence. Further, we will use a hypothesis generating approach to identify HPV types that are negatively associated and indicate the potential for HPV competition and warrant further investigation.

Specific Aim #2. To determine the associations between prevalent infections with vaccine-relevant HPV types and acquisition of other HPV infections over 24 months.

Aim 2.1. To describe the patterns of HPV acquisition among men with type-specific HPV infections at baseline to men without specific HPV types at baseline.

Aim 2.2. To determine if infection with one of six vaccine-relevant HPV type is associated with future acquisition of other HPV type infections, for all high-risk HPV types in pair-wise combinations.

Hypothesis. We hypothesize that men with baseline infections with vaccine-relevant HPV types will be more likely to acquire additional HPV types over follow-up as compared to individuals without baseline infections.

III: METHODS

Parent study: randomized control trial of male circumcision to reduce HIV

A randomized control trial of male circumcision was conducted from 2002-2007 in Kisumu, Kenya to assess the effectiveness of male circumcision to reduce the incidence of HIV infection (Grant number U01-A150440, P.I. Dr. Robert Bailey)[67]. The study was an unblinded randomized trial with two arms: the circumcision arm (intervention arm) and the delayed circumcised arm (control arm). Recruitment began in February, 2002, and enrollment was completed in September, 2005. Local newspapers, radio shows and street performances were used to disseminate information about the study. Potential participants were recruited from STI clinics, HIV voluntary testing and counseling centers, workplaces, and community organizations that serve unemployed and less-educated young men. At the first screening visit, potential participants were seen by trained counselors to determine if they met the study inclusion criteria, below.

Inclusion criteria:	**Exclusion criteria:**
Consent to participate	Foreskin covers less than half of the glans
Uncircumcised	Hemoglobin less than 90 g/L
HIV seronegative	Hemophiliac or other bleeding disorder
Sexually active	High prothrombin time index
Age 18–24 years	Other medical condition contraindicating surgery
Resident of Kisumu district	Absolute indication for circumcision
No plans to relocate for at least 2 years	

Eligible participants were invited for a second screening visit where they provided their written informed consent and were enrolled in the trial. At the next study visit (referred to as the baseline or randomization visit), participants were interviewed to obtain information on socio-demographic status, health, and sexual behavioral. Study nurses recorded participants' medical history and conducted a medical exam to collect blood and other biological samples. At this visit, men were assigned to either the intervention or the control arm based on randomly permuted blocks of size 10 and 20, within age-groups of 18–20 years and 21–24 years, to ensure approximately equal age distributions in the intervention and control arms. Men randomized to the circumcision arm were immediately scheduled for surgery; those randomized to the control arm were asked to remain uncircumcised until the end of the study, at which time they were offered circumcision.

Study participants were followed at 6 month intervals for a total of 24 months after randomization (all visits: 1) baseline/randomization, 2) 6 months, 3) 12 months, 4) 18 months, 5) 24 months). At each visit, a behavioral risk assessment, HIV testing and counseling, and STI testing and treatment were conducted. All men were counseled to reduce their risk for HIV and other STIs by consistently using condoms and reducing numbers of sex partners. During the medical exam at each visit, the following specimens were collected: blood for rapid HIV, herpes simplex virus type 2 (HSV-2) and syphilis (rapid plasma reagin) antibody testing; urine for nucleic acid testing for *N. gonorrhoea* and *C. trachomatis,* and *T. vaginalis* culture; a genital swab from men with urethral discharge for *N. gonorrhoea* and *T. vaginalis* culture, and *N. gonorrhoea* and *C. trachomatis* polymerase chain reaction (PCR); and a genital sample from men with genital ulcers for *H. ducreyi* culture, and *H. ducreyi*, syphilis and HSV-2 PCR testing.

Nested cohort study: natural history of HPV infections in men

Within the RCT is a nested cohort study of the effect of male circumcision on the risk and natural history of penile HPV infection among young, sexually active men in Kisumu, Kenya (Grant number R01 CA114773-04, P.I. Jennifer S. Smith)[32, 68, 69]. The aims of the nested study are to characterize the natural history of HPV infections in uncircumcised males, and to assess the effectiveness of male circumcision in reducing penile HPV incidence and HPV persistence. Following the design of the parent RCT, penile samples for HPV DNA detection and viral load were collected at baseline for all eligible males, and at biannual study visits through 24 months of follow-up for all men enrolled in the RCT and randomized to either the intervention or control arm.

Collection of penile samples for HPV DNA detection

Men who consented to the nested HPV study had penile exfoliated cells collected from two separate anatomical areas at each study visit during the medical examination. First, using a pre-wetted Dacron swab, exfoliated cells were collected by rubbing the external penile shaft vigorously a total of eight times with sufficient pressure in order to ensure that an adequate number of cells were collected. For uncircumcised men, this specimen also included a sample from the outer, external surface of the foreskin. Immediately following sample collection, the swab from external penile shaft and foreskin (referred to as the shaft specimen), was placed in one15mL tube containing Tris buffer. Next, with a different pre-wetted swab, cells were collected by circling the urethral orifice 2-3 times; by sampling back and forth from the top to

the bottom of the glans in a circular motion; and by rotating the swab three times completely around the circumference of the coronal sulcus. For uncircumcised men, an additional sample was taken using the same swab from the inner foreskin tissue. Immediately following sample collection, the swab of cells from the urethral orifice, glans, coronal sulcus, and inner foreskin tissue (referred to as the glans specimen) was placed in a second 15mL tube with Tris buffer. Both specimen tubes were sent directly to the Universities of Nairobi, Illinois and Manitoba (UNIM) lab to be processed and stored at -80 degrees.

HPV DNA laboratory detection

Samples were shipped to the Vrije Universiteit Medical Center in Amsterdam, Netherlands where they were evaluated for DNA quality by beta (β)-globin specific PCR using $BGPCO_3$ and $BGPCO_5$ primers [70]. HPV DNA positivity was assessed on all samples, regardless of β-globin positivity, using GP5+/6+ PCR and an enzyme immunoassay (EIA) read-out system with two HPV oligoprobe cocktails that detect 44 HPV types [70, 71]: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 (classified as HR-HPV), and 6, 11, 26, 30, 32, 34, 40, 42, 43, 44, 53, 54 55, 57, 61, 64, 67, 69, 70, 71 (equivalent to CP8061), 72, 73, 81 (equivalent to CP8304), 82 (IS39 and MM4 subtypes), 83 (equivalent to MM7), 84 (equivalent to MM8), 85 (cand85), 86, 89 (cand89 equivalent to CP6108), and JC9710 (classified as LR-HPV). For the full laboratory protocol of PCR methods, see Appendix Document 1. GP5+/6+ PCR positive samples were then subjected to HPV genotyping by reverse line blot hybridization (RLBH) [70]. HPV types detected by EIA but not by RLBH genotyping were designated as HPV-X, indicating a type, subtype, or variant not detectable with RLBH, and were not included in either the HR-HPV or LR-HPV groups. For each line blot, PCR products of positive and negative controls were included and the procedure was repeated if either one of these controls gave inappropriate

results. In addition to including positive and negative controls in each PCR and detection step, reproducibility of the test results was monitored periodically by randomly retesting 5% of samples. This highly specific PCR-EIA detection system with GP5+/6+ consensus primers has been shown to have lower rates of cross-hybridization between HPV types[58, 70] but is also relatively less likely to detect specific HPV types or multiple infections as compared to other detection methods[72, 73].

Data analysis methods

Overview

Our analyses aimed to answer: What is the distribution of HPV genotypes and number of concurrent infections among men? Are men without vaccine preventable HPV types 16, 18, 6, 11 more or less likely to have a concurrent infection with other specific HPV types?

Are individuals without vaccine-relevant HPV types 16, 18, 31, 45, 6 or 11 at baseline more or less likely to acquire other high-risk HPV types compared to individuals with these HPV type infections at baseline?

Sample population

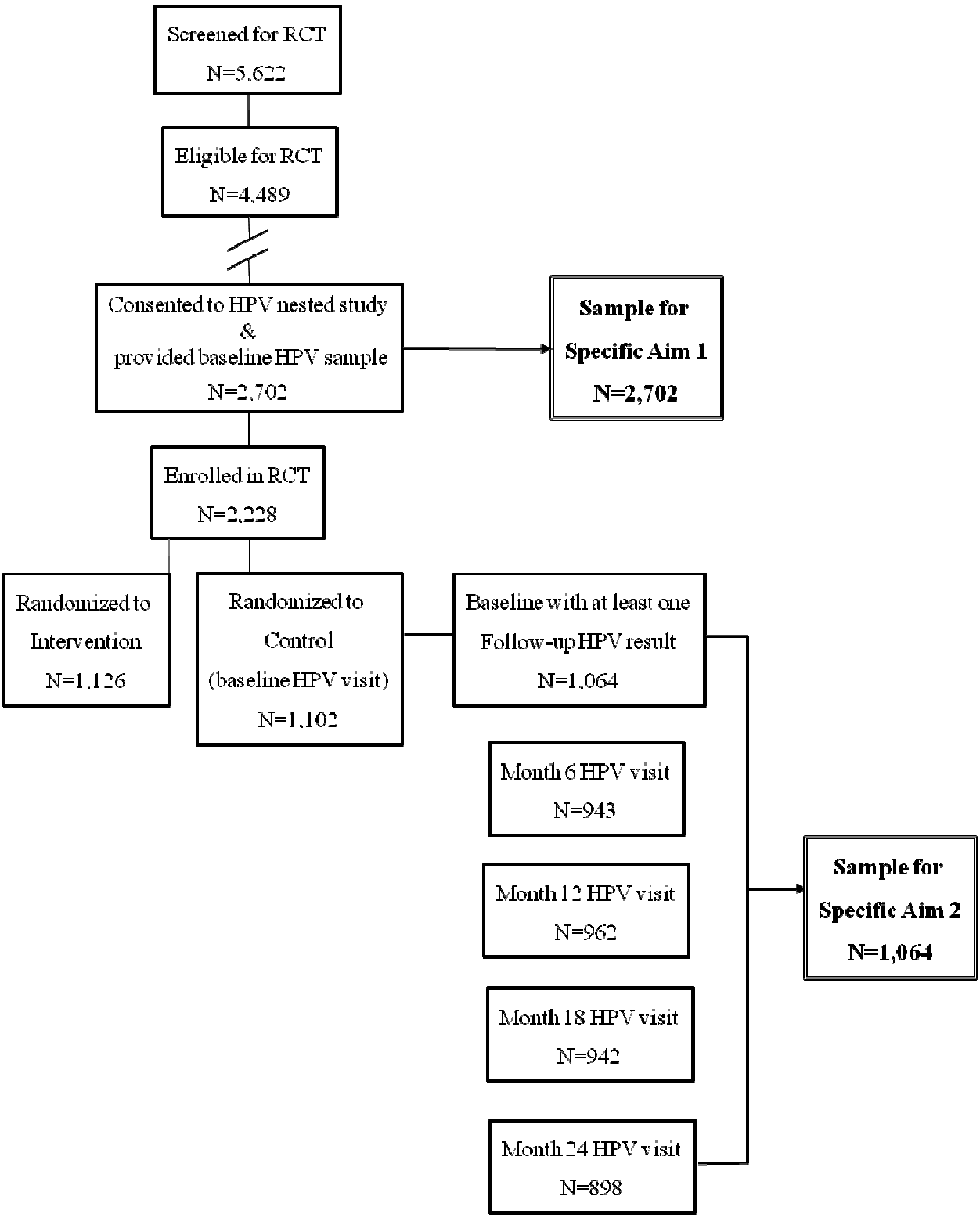
Men who were eligible for inclusion in the RCT (4,489 of 5,622 screened), and who consented to the HPV nested study and provided a penile sample at baseline (2,702 of 4,489) made up the sample population for analysis of study aim #1 (Figure 3.1). Men in this sample were HIV-negative, uncircumcised and were age 17-28 years. The study population for specific aim #2 were men who consented to the HPV nested study (2,702 of 4,489), who were enrolled in the RCT (2,228 of 2,702), who were randomized to the control arm (1,102 of 2,228) and who

had HPV DNA samples at baseline and at least one follow-up visit (1,064 of 1,102). Men in this sample were age 18-24 years and fully met all RCT inclusion criteria.

Categorization of HPV DNA results for analysis

For both specific aims #1 and #2, one of 44 HPV types was considered the exposure type and one of the other 43 HPV types was considered the outcome type, in order to determine the pair-wise associations between HPV types. All analyses were based on pooled HPV status rather than separate glans and shaft site-specific HPV status. This is because the aims relate to HPV vaccination that provides overall not site-specific protection against HPV infection. Furthermore, it is likely that an HPV type detected in the glans is also present on the shaft but it is less likely to be detected because of fewer exfoliated cells per sample[15]. In addition, main analyses included HPV DNA results regardless of detectable β-globin. As summarized in the results of previous publications from this cohort[32] and in the results section below (Chapter 6), approximately 70% of samples tested β-globin positive. The prevalence of HPV did not differ between β-globin positive and negative samples. HPV infections, rather than men, were treated as the unit of analysis because men could be simultaneously infected with multiple HPV types at any given time. Infections that could not be genotyped by RLBH were not included in either the HR-HPV or LR-HPV groups.

Figure 3.1. Study design and sample population for dissertation aims



Analysis of specific aim #1

Exposure and outcome definitions

Specific aim #1 focused on cross-sectional associations between the four vaccine-preventable HPV types (defined as 16, 18, 6, and 11) and all other 41 HPV types in pair-wise combinations: HR-HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and LR-HPV 26, 30, 32, 34, 40, 42, 43, 44, 53, 54 55, 57, 61, 64, 67, 69, 70, 71, 72, 73, 81, 82, 83, 84, 85, 86, 89, JC9710, and HPV-X. In addition, the distribution and correlates of multiple versus single HPV infections were described. A single infection is defined as baseline HPV DNA positivity to any one single HPV type in the glans and/or the shaft. For example, if HPV-16 was detected in the glans but not the shaft, or if HPV-16 was detected in both the glans and the shaft, an infection was classified as a single type infection. A multiple infection is defined as the detection of two or more different HPV types in either the glans or the shaft combined, regardless of the HPV types. For example, if HPV-16 was detected in the shaft and HPV-35 in the glans, or if HPV-16 and HPV-35 were detected in the glans and no HPV was detected in the shaft, an infection was classified as multiple type infection.

Missing data

All men included in the cross-sectional analysis of aim #1 provided a baseline HPV DNA sample (N=2,702). However, covariate data were missing (see Table 3.1 below for a detailed list of missing data). We did not impute missing values for covariates in the analysis of correlates of multiple infections but rather proceeded with a complete-case analysis since less than 5% of participants had incomplete data. Because potential confounding variables were only included in the first stage Bayesian models to calculate the adjusted prior

probabilities, missing data did not limit men from entering the hierarchical logistic regression analyses of HPV type associations (N=2,702).

The observed vs. expected number of HPV types

The distribution of the number and types of HPV detected were described (see Appendix Table 1 for the distribution of HPV types detected in single vs. multiple HPV infections, and Appendix Figure 1 for a graphical representation of HR vs. LR types in multiple and single infections, and of the number of HPV types stratified by type-specific HPV infection at baseline). The observed number of men with 0, 1, 2, 3, 4, 5, and 6 or more concurrent HPV types detected was compared to the frequency that would be expected under the assumption that each HPV infection is independent of all others. For each man, infection with each of the 45 possible HPV types was simulated by random generation of a binary variable with the probability of infection equal to the observed prevalence of that type in the study population[42]. The expected frequencies of each number of HPV infections were then calculated as the average frequency over the 1,000 stochastic simulations of 2,702 observations. The observed prevalence of the 14 HR-HPV types was used to simulate the expected number of infections with only high-risk HPV types. Data simulation, as opposed to multiplication of the marginal probabilities, was a much more efficient method to determine the expected number of infections. For example, in order to determine the expected frequency of infection with any two HPV types without data simulation, 990 probabilities would have to be calculated (e.g. 45 chose 2 to determine the probability of being positive for type 1-2 and negative for all other types, plus the probability of being positive for types 3-4 and negative for all other types, etc).

Two-sided p-values were calculated to determine if the observed frequency of 0. 1, 2, 3, 4,5, 6+ infections was less than or greater than the expected frequency if each infection was independent. For i=0 to 6+ HPV types, O_i was the observed number of men with i infections, and E_i was the expected number of men with i infections based on data simulations. For simulated the datasets j=1 to 1000, O_{ij} was the number of men with i infections in the j^{th} dataset. Thus, the two-sided p-value was calculated as proportion of simulated data sets where $|O_{ij}-E_i| >= |O_i-E_i|$.

Correlates of multiple HPV infections

Study covariates that were investigated as potential correlates of multiple HPV infections were identified in the previous literature on multiple infections in men and women and previous baseline analysis of this cohort[32, 37, 74]. The categorizations of the variables that we considered in our analysis are outlined below (Table 3.1).

Univariate logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for potential correlates of multiple versus single HPV infections. Multivariate logistic regression was used to estimate associations between each potential correlate and multiple HPV infections, simultaneously adjusting for all other potential correlates. These variables were also considered potential confounders in the analysis of HPV type associations, below.

The degree of collinearity between variables included in the multivariate model was assessed by examining the variance inflation factor (VIF) and tolerance (1/VIF) values. No VIF values exceeded the cut-off of 10 (equal to a tolerance value less than 0.1), which was a

value chosen to highlight variables likely to be a linear combination of other variables in the model[75].

Table 3.1. Covariates considered in univariate and multivariate models as potential correlates of multiple HPV infections

Study covariates	Missing	Model inclusion	Justification
Age (in years)	n=0	Continuous	Previous literature on prevalence and acquisition of HPV infections
Travel to Nairobi (6 months prior to baseline)	n=5	Binary (any/none)	Potential exposure to additional HPV types and relates to SES
Bathing frequency	n=14	Binary (daily/less than)	Baseline paper on risk factors, to represent male hygiene[a]
Number of partners (6 months prior to baseline)	n=23	Categorical (0-1 vs. 2+)	Previous literature and baseline paper on risk factors
Condom use (6 months prior to baseline)	n=129	Binary[b] (always, not always)	Previous literature and baseline paper on risk factors
N. gonorrhea	n=23	Binary (detected/not)	Baseline paper on risk factors and represents marker of sexual activity/sexual risk behaviors
C. trachomatis	n=24	Binary (detected/not)	Baseline paper on risk factors and represents marker of sexual activity/sexual risk behaviors

[a]Baseline paper on risk factors for HPV infection within this cohort (J. Smith, IJC, 2010)
[b] "Always" category includes men who report no sex in last 6 months

Semi-Bayesian methods for HPV type associations

Next, hierarchical regression analysis was used to obtain semi-Bayes estimates of the ORs[76] between the four vaccine-preventable HPV types and 41 other outcome HPV types, adjusting for potential confounders of the association between HPV types. When data are sparse, shrinkage methods, such as the one we used here, reduce the overall error in the set of

estimates as compared to maximum likelihood estimation (MLE). Prior information from the study data (in this analysis: the average association between all types) and from the literature (in this analysis: the range of expected odds ratios) are incorporated into these two-stage models[77-79].

The type-specific prior means for HPV-16, 18, 6 and 11, μ_j, where j = 1 to 4, were estimated from the data and their variability was propagated through hyperpriors. The μ_j are the average of the log odds ratio between the individual vaccine type and all 41 other HPV types, adjusted for age, travel to Nairobi (in 6 months), bathing frequency, number of sexual partner (in 6 months), consistent condom use (in 6 months), and current Gonorrhea and *C. trachomatis* infection. Multivariate logistic regression models for all 164 pair-wise combinations (4 exposure types by 41 outcome types) were conducted to obtain the individual adjusted ORs. Then, the crude averages of the ORs were calculated separately for each of four exposure types. Thus, the prior inputs for the semi-Bayesian model were: μ_{16}=0.6796, μ_{18}=0.9264, μ_6=0.6238, μ_{11}=0.7605. A hyperprior for the type-specific prior means was added to the models to express our uncertainty around the estimates of μ_j [78]. The hyperprior, μ_H, was normally distributed with a mean zero and a variance of 0.5. Next, we assumed that 95% of the log odds ratios should fall within a 5-fold range based on previous literature [41-43, 55, 60]. Thus, the prior variance for each μ_j, τ^2, was set equal to 0.169 in the main analysis, which was back calculated from the assumed OR range: $\tau^2 = \{[\ln(OR_U)-\ln(OR_L)]/3.92\}^2$. Model estimates are reported as the exponentiated posterior medians and 95% credible intervals, analogous to ORs and 95% CIs.

Semi-Bayesian logistic regression was implemented in SAS version 9.2 using the PROC MCMC (Markov Chain Monte Carlo) procedure. An example of the SAS code is

provided, which highlights model settings and parameterization (Appendix document 2). The models were set with 2,000 burn-in iterations, 2,000 tuning iterations and 80,000 MCMC iterations (excludes burn-in) with a thinning rate of 10 (only 1 out of every 10 iterations is kept). The final models settings were chosen based on a review of model fit and in accordance with current expectations in the literature (personal communication with Dr. Steve Cole: a review of PROC MCMC SAS coding, model diagnostics, and iteration settings). Representative sets of diagnostic plots (HPV-45 and HPV-52) for model convergence and fit are presented in Appendix Figure 2:

1. The stable mean of the trace plots indicates the Markov chain has converged and the full coverage (e.g. traces span the full range and are thick) of the plots indicates the models have good mixing.

2. The plots of autocorrelations of the posterior samples shows that the autocorrelations drop quickly and remain zero after 5-10 lags, which shows the model settings were sufficient.

3. The kernel density plots show the distribution for the estimates of the posterior marginal distributions for each parameter, which are a smooth, bell-shape, indicating a well-fit model.

Sensitivity analyses

1) The case of the bivalent HPV vaccine was also considered; analyses were conducted as outlined above except only two vaccine types, HPV-16 and 18, were considered

the exposure types, and the 43 other types, including HPV-6 and 11, were considered the outcome types (results presented in Chapter 4).

2) The precision of the estimates from the semi-Bayesian logistic regression is partially dependent on the value of the prior variance of the prior probabilities, τ^2. We explored the results obtained when setting τ^2 to 0.35, which reflects a 10-fold difference in the prior 95% confidence limits and 1.38, which reflects a 100-fold difference in the prior 95% confidence limits (results presented in Chapter 4).

3) Previous studies of HPV type associations and competition have used maximum likelihood estimation techniques. Therefore, to compare our results with other published studies, ORs of HPV type associations were also estimated using maximum likelihood logistic regression. These models were constructed in the same manner as the semi-Bayes models: the individual models for each of the 41 outcomes types contained the four exposure HPV types and the covariates age, travel to Nairobi, bathing frequency, number of sexual partner, consistent condom use, and current Gonorrhea and *C. trachomatis* infection (results presented in Chapter 4).

Strengths and limitations

Although the analytical methods for specific aim #1 improved upon previous studies, there are several limitations to highlight in order to properly interpret the results. First, there were no data on immunological susceptibility and no data on HPV types detected in female sexual partners. These unmeasured cofounders could affect HPV infection status and genotype distribution, and thus affect the observed associations between HPV types. Although we were able to adjust for several measured behavioral factors that are potential

confounding variables, the observed associations between HPV types are influenced by unmeasured factors that cause positive (e.g. shared risk factors and transmission routes) and negative (e.g. low prevalence of specific HPV types or potential type competition) associations between HPV types.

Using multiple models to draw conclusions regarding 164 pair-wise HPV type associations raises the problem of multiple comparisons, which can increase the probability of falsely concluding HPV types associated. On the other hand, the number of comparisons in our analysis is reduced compared to previous studies[58-61]. By including all four exposure types in each model, we not only adjusted for infection with the other HPV types but fewer models were needed to determine all pair-wise associations. The semi-Bayesian methods have increased power to detect differences between even rarer HPV types and reliably reduce the overall error in the set of estimates because of the information gained by incorporating prior probabilities into two stage modeling[77-79]. However, it is important to note that when the data are sparse, the prior information carries more weight in the regression analysis and the estimates are shrunk towards the grand mean, while estimates with more data will be less influenced by the prior probabilities.

Analysis of specific aim #2

Exposure and outcome definitions

Specific aim #2 was a prospective analysis that focused on the association between type-specific HPV infection at baseline and acquisition of additional HPV infections over 24 months of follow-up. The exposure of interest was a baseline infection with HPV-16, 18, 31, 45, 6, or 11. HPV types 16, 18, 6 and 11 were chosen as baseline types of interest due to

their inclusion in current generation HPV prophylactic vaccines. Given the relatively high prevalence of HPV 45 in adenocarcinoma and the potential for HPV cross-protection against HPV-31[10], we also investigated associations between HPV-31 and 45 and future HPV acquisition. The outcome was acquisition of any-HPV, HR-HPV and each 1 of 14 HR-HPV types over 24 months of follow-up (Table 3.2). In both the analysis of group and individual HPV type outcomes, type-specific HPV acquisition was defined as the detection of a new genotype at the current visit that was not detected at any of the previous study visits.

Table 3.2. Index and referent groups for the analysis of HPV acquisition

Baseline exposures of interest	Acquisition outcomes of interest[a]
Vaccine-relevant types	
HPV16+ *vs.* HPV16-	Any-HPR, HR-HPV, HPV-18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68
HPV18+ *vs.* HPV18-	Any-HPR, HR-HPV, HPV-16, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68
HPV31+ *vs.* HPV31-	Any-HPR, HR-HPV, HPV-16, 18, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68
HPV45+ *vs.* HPV45	Any-HPR, HR-HPV, HPV-16, 18, 31, 33, 35, 39, 51, 52, 56, 58, 59, 66, 68
HPV6+ *vs.* HPV6-	Any-HPR, HR-HPV, HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68
HPV11+ *vs.* HPV11-	Any-HPR, HR-HPV, HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68
Vaccine-relevant groups	
HPV16 and/or 18+ *vs.* HPV16 and 18-	HPV-31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68
HPV6 and/or 11+ *vs.* HPV6 and 11-	HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68
HPV16, 18, 6 and/or 11+ *vs.* HPV16, 18, 6, 11-	HPV-31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68

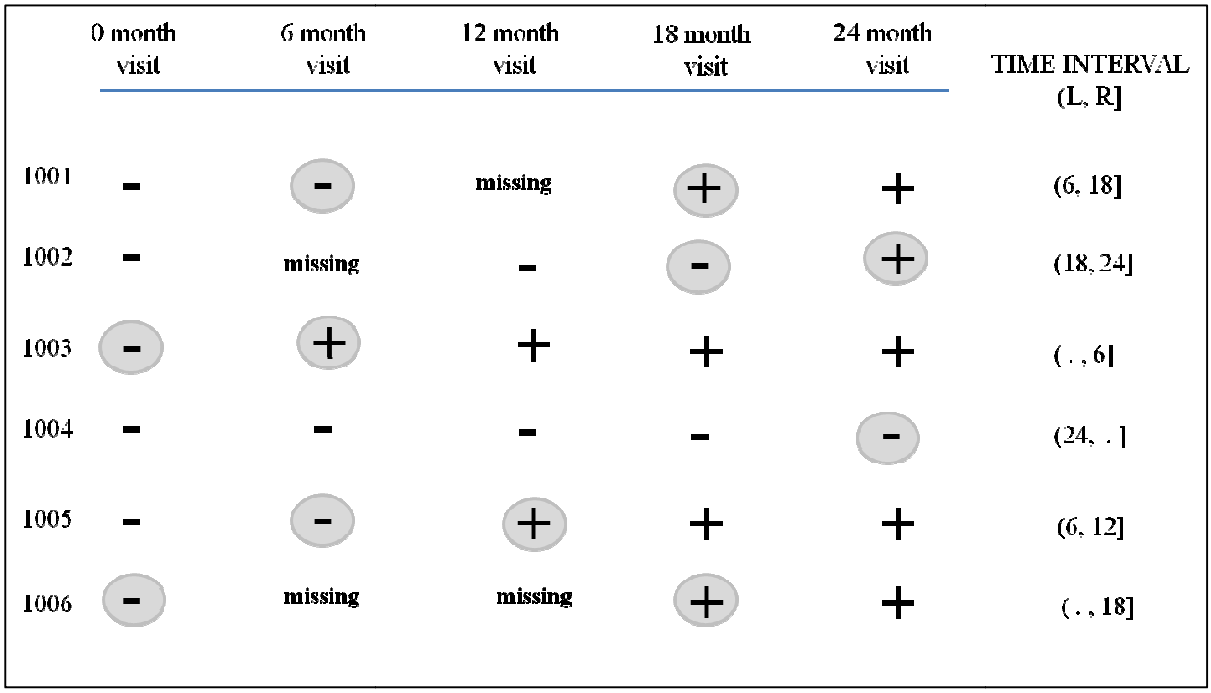
[a]Outcome group or type always excludes the exposure HPV type of interest in each analysis

Time to event: interval censoring methods

Interval censored survival methods for time to first acquisition for each HPV type were used because the actual time of acquisition was not directly observed; acquisition events are only known to have occurred between the last HPV negative visit and the first HPV positive visit[80]. For each HPV type where an event was not observed, the data were right-censored at the final study visit (Figure 3.2). If men crossed-over to the circumcision arm during follow-up before experiencing an event, they were right-censored at their last visit with HPV DNA results prior to their circumcision.

The intervals were used to accommodate missing visits where HPV DNA results were unavailable. If the visit(s) before the first HPV positive visit was missing, the interval was widened to accommodate the missing observation(s). For example, the left interval was the last HPV negative visit date and the right interval was the first HPV positive date, with the missing visit inside this interval. If HPV DNA results were missing in between two HPV negative visits, then the missing value was assumed to be HPV negative and it was not included inside the acquisition event interval.

Figure 3.2. Diagram of interval censoring scheme used in time-to-event analyses



Parametric survival models

Parametric frailty survival models were used to estimate the overall association between type-specific infections at baseline and acquisition of any-HPV and HR-HPV. Parametric frailty models account for the correlation between HPV types among men who acquire multiple infections[81] and allow for interval censored data. Survival times were assumed to follow a Weibull distribution, such that the model can be viewed as either an accelerated failure time model or a proportional hazards model[81, 82]. In the Weibull model,

$$h_{ij}(t; \boldsymbol{x}|\xi_i) = \gamma(t^{(\gamma-1)})\exp(\beta_0 + x_{ij}\beta + \xi_i),$$

for the i^{th} participant and the j^{th} HPV genotype, where γ is the shape parameter,and ξ_i is the random frailty effect, which is assumed to be normally distributed with a mean of zero

exp(β) represents the adjusted hazard ratio (aHR) and exp($\frac{1}{\gamma}$ β) represents the adjusted median survival ratio (aMSR) for men with a baseline HPV type compared to men without that baseline type. aHRs less than 1.0 indicates a lower rate of HPV acquisition among men positive for a specific HPV type compared to men negative for that same type at baseline, adjusted for potential confounding variables. Similarly, the aMSRs compare the median survival time among HPV infected versus uninfected men at baseline, adjusted for potential confounding variables.

Parametric survival models without the random effects frailty term were used to estimate the aHRs for the associations between prevalent HPV types and acquisition of each one of the 14 individual HR-HPV types. In the Weibull model:

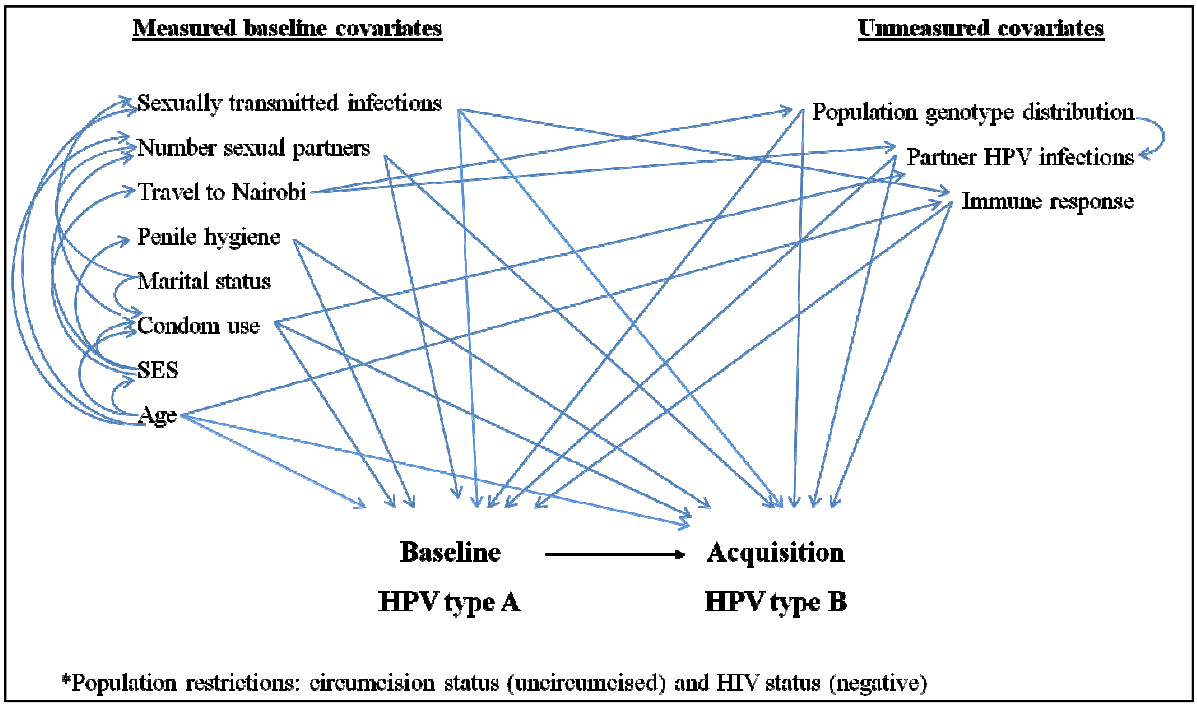
$$h(t;\boldsymbol{x}) = \gamma(t^{(\gamma-1)})\exp(\beta_0 + \boldsymbol{x\beta}),$$

γ is the shape parameter, $\boldsymbol{x}$ is a vector of covariate values and $\boldsymbol{\beta}$ is a vector of coefficients

Variables were identified as potential confounders of the association between baseline HPV type and time to acquisition of additional HPV types through an extensive review of the literature. A directed acyclic graph (DAG) was used determine the minimally sufficient set of adjustment variable to best control for confounding (Figure 3.4). All models of HPV acquisition were adjusted for potential confounding variables, including baseline measurement of age (continuous centered at the median age of 20 years; no missing), male hygiene (daily vs. less than daily bathing frequency; missing=13), number of sexual partners in 6 months prior to baseline (0-1 vs. ≥2; missing=4), consistent condom use in 6 months

prior to baseline (self-reported always vs. less than always condom use; missing=115) and the presence of *C. trachomatis* (baseline STI shown in aim #1 to be associated with multiple HPV infections; missing=12). Covariate data was complete for ~90% of the sample population so a complete case analysis approach was used.

Figure 3.4. Directed acyclic graph of the associations between baseline HPV types and time to acquisition of other HPV types



Survival curves to assess model fit and describe patterns of HPV acquisition

Survival curves were used to graphically display the pattern of HPV acquisition by HPV-16, 18, 31, 45, 6 and 11 DNA status at baseline. The survival function, which describes the probability that an HPV infection has not been acquired as a function of time, was estimated using the nonparametric maximum likelihood estimator (NPMLE)[83, 84], which

is the general form of the Kaplan-Meier estimator for interval censored data. We used the published SAS macro %ICE, which is appropriate for data that are arbitrarily censored at varying times and with varying interval widths[80]. This macro produces survival function estimates and confidence limits for one or more groups using the iterative convex estimator (ICE) of Wellner and Zhan[85]. This method has been shown to be an efficient method for computing the NPMLE of the survival function with interval-censored data. We chose to graph the cumulative distribution function, which is one minus the survival function (F=1-S), so the figures represent the cumulative probability of HPV acquisition.

To assess model fit, the NPMLE of the survival function for each baseline HPV type were plotted along with the parametric estimates of the survival function estimated by the Weibull frailty model (Appendix Figure 3). The survival functions were not adjusted for other covariates, however, the frailty models do account for multiple HPV acquisitions. A good model fit is indicated by overlapping survival curves, with similar shape and values, from both the nonparametric and parametric models. The absolute values of the probability of HPV acquisition are consistently slightly lower for the parametric MLE than the nonparametric estimates. However, both sets of curves are monotonic and have a similar shape, showing an increase in HPV acquisition over time. Curves from both the parametric models with an assumed Weibull distribution for the survival time and the nonparametric models indicate the same general conclusions regarding the relative probabilities of acquisition among men with as compared to without baseline infections with each of the six HPV types (HPV-16, 18, 31, 45, 6, or 11).

Sensitivity analyses

1) The main analysis of any-HPV and HR-HPV acquisition was conducted using parametric survival models with a random effects frailty term and adjustment for several confounding variables. Therefore, models without the frailty term and adjustment variables were also conducted for any-HPV and HR-HPV acquisition (Appendix Table 2). In general, there was little difference between adjusted and unadjusted aHRs for HPV acquisition. The precision of estimates from the models that included the frailty term, which took into account the possible correlation between HPV among men with multiple infections, was reduced compared to the equivalent model without the frailty term. This implies that each additional infection contributed less information to the model than if all infections were considered independent, as in the non-frailty models.

2) Men with no HPV for prolonged periods of time may differ from men who have HPV over a two year period, either behaviorally or immunologically. Therefore, we conducted additional frailty modeling analyses restricted to just men who had at least on HPV type detected at least once (results presented in Chapter 5).

3) In construction of the censoring intervals, we assumed that a missing HPV DNA result that was between two HPV negative visits, was also HPV negative (limited case of last value carried forward). An alternative choice would be to use imputation methods to assign a value to the missing HPV data. Data imputation would assign some missing values to be HPV negative and some to be HPV positive based on the pattern of covariate data. The overall effect on our estimate of HPV acquisition would be somewhere in between assuming all missings were HPV negative or positive. Therefore, to better understand our HPV-negative assumption, and to gage the utility of imputation methods, we changed the missing

value to HPV positive and re-analyzed the data. The point estimates and confidence intervals from the sensitivity analysis were very similar to the results of the original analysis (Appendix Table 3). Therefore, we proceeded with our original method and did not use data imputation methods.

Strengths and limitations

This analysis was limited to men randomized to the control arm. Therefore, the sample size is smaller than full cohort and it's possible that the results are not generalizable to circumcised men. The smaller samples size could result in reduced power to detect differences by baseline status, especially for rarely acquired HPV types. In addition, the analysis of aim #2 also has the problem of multiple comparisons since only one outcome type or group is included in each model. However, it was important to examine all high-risk pair-wise associations between vaccine-relevant HPV types and future HR-HPV acquisition, as these relate to risk of anogenital cancers and the impact of HPV vaccination. This analysis focused only on HPV acquisition but HPV persistence may also differ by HPV type. The association between type-specific HPV infections and HPV persistence will be examined in a future analysis. Splitting HPV acquisition and HPV persistence into separate papers allows us to explore both topics in-depth with numerous HPV group and type-specific analyses.

We used novel survival methods to account for correlation between types among men who acquire multiple HPV infections in the analysis of grouped outcomes (e.g. any-HPV and HR-HPV). Another strength of our analysis is the method of calculating time-to-event. We chose to use interval censoring techniques because it requires fewer assumptions regarding when the event happened, as opposed to test date or mid-point assumptions. Interval censoring methods are appropriate for time-to-event determination for epidemiologic data,

particularly for studies with biannual testing intervals for an infection as dynamic HPV. In addition, by using interval-censoring, the only assumption we had to make regarding missing data was that a missing value in between two negative tests was HPV negative since all other missed visits were included in the acquisition interval. This method required fewer and potentially more realistic assumptions than carrying the last value forward for all missing data. Also, if we had instead chosen to censor individuals at the first missed visit, we would have reduced our sample size and power; a lot of men who were missing at 6 months returned for study visits at 12, 18, and 24 months.

IV: MULTIPLE HUMAN PAPILLOMAVIRUS INFECTIONS AND TYPE COMPETITION AMONG KENYAN MEN

Abstract

Background: There is little information on multiple HPV infections and the potential for type competition in men, yet competition may impact the type-specific efficacy of HPV vaccination.

Methods: To assess the potential for HPV type competition, adjusted odds ratios for pair-wise combinations of prevalent HPV type infections were estimated using semi-Bayesian methods among 2,702 uncircumcised, HIV-seronegative men in Kisumu, Kenya. The observed numbers of HPV types detected were compared to the expected number, which was simulated under the assumption of independent infections.

Results: Half of all men were HPV positive, of whom 57% had multiple HPV types. We observed men without HPV infection and with four or more HPV types more often than expected if infections were independent. No negative associations between individual HPV types were observed. HPV types 31, 39, 56, 58, and 59 were positively associated with both carcinogenic vaccine types HPV-16 and 18 (two-sided p-value <0.05).

Conclusions: Men who were HPV infected were likely to test positive for more than one HPV type. Cross-sectional associations between individual HPV types were positive and did not appear to be type-specific. Thus, we did not identify HPV types that are candidates for potential HPV type competition in men.

Introduction

Human papillomavirus (HPV) infection is the main cause of cervical cancer in women[3, 86], and responsible for other genital cancers, including anal and penile carcinoma in men. Co-infection with multiple HPV types is common and observed in 20-73% of HPV infected males[14, 32-38, 52]. Multiple HPV type infections have been associated with acquisition of other HPV types and increased HPV persistence in men[35], and cervical precancerous lesions in women[46-48].

There are currently two FDA-approved HPV vaccines that provide protection against HPV-16 and 18 [9-11] or HPV-16, 18, 6 and 11 [5-7]. Nearly 70% of cases of cervical cancer have been attributed to infection with oncogenic HPV types 16 and 18[1, 8] and low-risk HPV types 6 and 11 are responsible for the 90% of genital warts. HPV type co-infections could affect the population-based impact of HPV vaccination in both young women and men due to potential HPV type competition and subsequent type replacement[40, 41]; that is, an increase in the prevalence of non-vaccine HPV types in the population when vaccine-preventable HPV types are reduced or eliminated[51]. Type competition may result from some yet unknown biological mechanism, whereby infection with one HPV type inhibits the acquisition or persistence of other HPV types [35, 42]. In contrast, if infection with a specific HPV type facilitates the acquisition or persistence of other HPV types, it is possible that when one HPV type is prevented by vaccination, the other type might be reduced in the population.

If HPV types do compete, this will be reflected in the population as a low probability of co-infection with two specific HPV types. However, there are several reasons why, in addition to the possibility of type competition, that two HPV types may be less likely to

occur together within a multiple infection. The prevalence of HPV types detected in a sample population are dependent on the HPV type distribution in the general population in that geographical area, as well as the HPV types circulating within their networks of sexual contacts. In addition, observed positive associations between HPV types may be due to the common transmission route and risk factors for all HPV types, such as age[49-51], condom use[37], circumcision status[34, 52], and lifetime[37, 53, 54] or recent[50] number of sexual partners. Thus, any association between individual HPV types reflects a combination of exposure to HPV, the type distribution among sexual contacts, and immunological and behavioral characteristics[35, 42, 55].

To date, there are no detailed studies of multiple HPV infections and type competition in an African population, where the HPV genotype distribution may differ from those in North America, Europe or South America[65]. Given the recent approval of HPV prophylactic vaccination in the United States for young men[66], data on HPV co-infections in men and their female sexual partners are needed to assess the potential for future HPV type replacement. Using data from a randomized control trial of male circumcision in Kisumu, Kenya[67, 69], we investigated the association between vaccine-preventable HPV types (16, 18, 6 and 11) and 41 other HPV types using semi-Bayesian methodology.

Methods

Study population and design

From February 2002 to December 2006, a randomized controlled trial (RCT) was conducted in Kisumu, Kenya to determine the effectiveness of male circumcision in reducing HIV incidence[67]. Male participants were recruited from sexually transmitted infection

(STI) clinics, workplaces, and community organizations. To be included in the RCT, males were required to be between 18-24 years old, uncircumcised, HIV seronegative, sexually active, and have a hemoglobin level of 90 g/L or more.

An observational cohort study of the effect of circumcision on the natural history of HPV infection was nested in the RCT[32, 68, 69]. The present analysis is a cross-sectional study at the baseline visit of uncircumcised, HIV-seronegative men who were eligible for participation in the main RCT (n=4,489) and who consented to HPV testing (2,705)[32]. In the present analysis, three men were excluded due inconsistent records in the database. The final population for this analysis includes 2,702 men who provided penile samples and information on sexual risk factors at baseline, of which 2,228 were enrolled in the RCT. The study protocol was approved by the Institutional Review Boards at all collaborating Institutions.

Medical examination and sample collection

After participants provided informed consent, trained male interviewers administered a standardized questionnaire on sociodemographic characteristics, medical conditions, and sexual behavior. A trained physician or clinical officer conducted a physical exam during which a genital examination was conducted to visually inspect for ulcers and warts. Blood was collected for HSV-2 (Kalon Biological, Ltd) and syphilis testing (Becton Dickinson; RPR with TPHA confirmation). Urine samples were collected for PCR detection of *N. gonorrhea* and *Chlamydia trachomatis* (Roche Diagnostics).

Exfoliated penile cells were collected from two anatomical sites using two pre-wetted Dacron swabs: i) the shaft and external foreskin tissue (the shaft specimen) and ii) the glans,

coronal sulcus and inner foreskin tissue (the glans specimen)[69]. The penile swabs were placed in separate 15-mL centrifuge tubes containing 2-mL Tris buffer 0.01M and processed on the day of collection. All samples were sent to the Department of Pathology at the VU University Medical Center for laboratory detection of HPV DNA.

Detection of HPV DNA

Laboratory detection. HPV DNA testing was performed on uncircumcised men at the baseline study visit. DNA was isolated from exfoliated penile cell samples[70, 71] and the presence of human DNA was evaluated by beta-globin-specific polymerase chain reaction (PCR), followed by agarose gel electrophoresis. The presence of HPV DNA was assessed by GP5+/6+ PCR, followed by hybridization of PCR products using an enzyme immunoassay readout with 2 HPV oligoprobe cocktail probes that together detect 44 HPV types. Subsequent HPV genotyping was performed by reverse line blot (RLBH) hybridization of PCR products, as described elsewhere[70, 71]. HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 were classified as high-risk (carcinogenic) HPV types. Low-risk types included 6, 11, 26, 30, 32, 34, 40, 42, 43, 44, 53, 54 55, 57, 61, 64, 67, 69, 70, 71 (equivalent to CP8061), 72, 73, 81 (equivalent to CP8304), 82 (IS39 and MM4 subtypes), 83 (equivalent to MM7), 84 (equivalent to MM8), 85 (cand85), 86, 89 (cand89 equivalent to CP6108), and JC9710. Types considered vaccine-preventable are HPV-16, 18, 6 and 11, whereas all others are non-vaccine preventable HPV types. HPV types detected by enzyme immunoassay but not genotyped were designated as HPV-X, indicating a type, subtype, or variant not detectable with RLBH probes.

Definition of multiple infections. HPV DNA detection methods were carried out on the shaft and glans samples separately. Given that the aims of the present analyses relate to evaluating the potential for HPV type replacement following population-based HPV vaccination and not site-specific infection, we present pooled HPV results for the shaft and glans specimens. A single infection is defined as HPV DNA positivity to any one single HPV type in the glans, the shaft, or both sites. A multiple infection is defined as the detection of two or more different HPV types in either the glans or the shaft combined. For example, if a man was HPV-16 positive in the shaft and HPV-35 positive in the glans, or if a man was HPV-16 and HPV-35 positive in the glans and HPV negative in the shaft, in either case the man was classified as having multiple infections.

Statistical analysis

Distribution of number of HPV genotypes. The observed number of men with 0, 1, 2, 3, 4, 5, and 6 or more concurrent HPV type infections was compared to the frequency that would be expected under the assumption that each HPV infection is independent of all others. Infection with each of the 45 possible HPV types was simulated for each man by random generation of a binary variable with the probability of infection equal to the observed prevalence of that type in the study population. Expected frequencies for each number of concurrent HPV infections were calculated as the average frequency over 1,000 stochastic simulations of 2,702 observations[42]. For HR-HPV simulations, the observed probabilities of the 14 HR-HPV types were used to simulate the expected number of infections with only high-risk HPV types. As done previously[32], all analyses included males who were HPV

positive regardless of beta-globin positivity and were conducted using SAS version 9.2 (Cary, NC).

Correlates of multiple HPV infections. Univariate logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for potential correlates of multiple versus single HPV infections. Variables were identified *a priori* as potential correlates of multiple HPV infection based on the previous literature[32, 35, 37]. Multivariate logistic regression was used to estimate associations between each potential correlate and multiple HPV infections, simultaneously adjusting for all other potential correlates.

HPV type associations. We used hierarchical regression analysis to obtain semi-Bayes estimates of the ORs [76] between the four vaccine-preventable HPV types and 41 other outcome HPV types, adjusting for the potential correlates of multiple HPV infections. Shrinkage methods, such as the one we used here, reliably reduce the overall error in the ensemble of estimates, by incorporating prior information from the study data and the literature[77-79]. HPV-16, 18, 6 and 11 type-specific prior means, μ_j, where j =1 to 4, were estimated from the data and their variability was propagated through hyperpriors. The μ_j are the average of the log odds ratio between the individual vaccine type and all 41 other HPV types, adjusted for age, travel to Nairobi (in 6 months prior to baseline), bathing frequency, number of sexual partners (in 6 months prior to baseline), consistent condom use (in 6 months prior to baseline), and current Gonorrhea and *C. trachomatis* infections. The prior variance for each μ_j, τ^2, was set equal to 0.17 because we assumed that 95% of the log odds ratios should fall within a 5-fold range based on previous literature [41-43, 55, 60], which corresponds to a variance of $[\ln(5)/3.92]^2 = 0.17$.

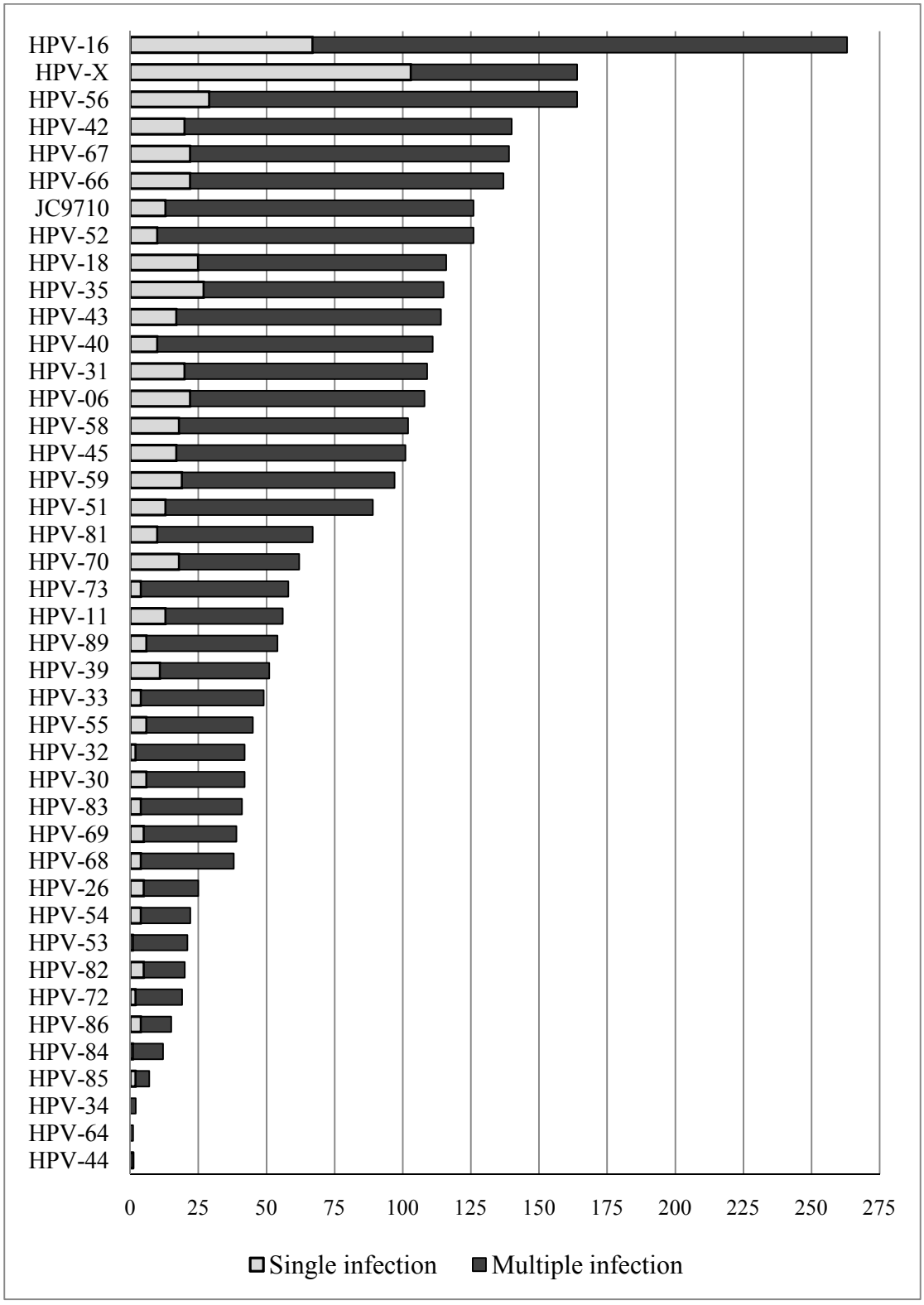
Model estimates are reported as the exponentiated posterior medians and 95% credible intervals, analogous to ORs and corresponding 95% CIs. Potential evidence of HPV type non-independence is an OR estimate less than 1.0: the odds of a non-vaccine preventable type are lower in men with a vaccine-preventable HPV type compared to men without a vaccine-preventable type.

Sensitivity analysis. The case of the bivalent HPV vaccine was also considered; analyses were conducted as outlined above, except HPV-16 and 18 were considered exposure types, and the 43 other types, including HPV-6 and 11, were considered outcome types. Further, we explored results obtained when setting tau^2 to 0.35 or 1.38, reflecting a 10- and 100-fold prior 95% confidence limit. For comparison, maximum likelihood estimates of HPV type associations are also presented (online Appendix Table 1).

Results

Among 2,702 men, with a median age of 20 years (range: 17-28), 51% of men were HPV positive (n=1,379). A single HPV type was detected in 22% (n=592) and multiple HPV types were detected in 29% (n=787) of men. The five most prevalent types overall were HPV-16 (n=263; 10%), HPV-X (n=164; 6%), HPV-56 (n=164; 6%), HPV-42 (n=140; 5%), and HPV-67 (n=139; 5%). The five most prevalent types within multiple infections were HPV-16 (n=196; 25%), HPV-56 (n=135; 17%), HPV-42 (n=120; 15%), HPV-67 (n=117; 15%), and HPV-52 (n=116; 15%)(Figure 4.1). All HPV types were more likely to be detected as multiple infections rather than single infections, with the exception of untypeable infections (HPV-X: 37% multiple, 63% single).

Figure 4.1. HPV genotype distribution of multiple and single infections, in order of descending prevalence



The number of HPV types detected within an individual ranged from 0 to 11 infections. The observed frequency of zero HPV infections and infection with four, five, and six or more HPV types were higher than expected under the assumption that infections are independent (two-sided p-value<0.01) (Table 4.1). Frequencies of one or two HPV types were less than expected (p<0.01). The distribution of observed high-risk genotype infections was similar to infection with any HPV genotype.

Table 4.1. Comparison of the observed and expected number of human papillomavirus (HPV) types detected

Number of HPV genotypes	Any HPV			HR-HPV		
	Observed	Expected	*p-value*[a]	Observed	Expected	*p-value*[a]
0	1323	832.9	<0.01	1749	1494.3	<0.01
1	592	1002.1	<0.01	571	909.4	<0.01
2	349	581.1	<0.01	230	251.5	0.15
3	194	214.6	0.15	103	41.6	<0.01
4	111	57.3	<0.01	34	4.8	<0.01
5	68	11.9	<0.01	11	0.4	<0.01
≥6	65	2.2	<0.01	4	0.0	<0.01
Mean (SD)	1.15 (1.60)	1.15 (1.05)		0.58 (0.96)	0.58 (0.74)	

Abbreviations: HR-HPV (high-risk human papillomavirus); SD (standard deviation)
[a] Two-sided p-value calculation: For i=0 to 6+ types, let O_i be the observed number of men with i infections and let E_i be the expected number of men with i infections (based on 1000 simulations) assuming independence. For simulated data sets j=1 to 1000, let O_{ij} be the number of men with i infections. Then for i=0 to 6+ types, the two-sided p-value was calculated as proportion of simulated data sets where $|O_{ij}-E_i| >= |O_i-E_i|$

Multiple HPV infections were more common among men who were younger, bathed less often than daily, and had chlamydia infection. Men were less likely to have multiple versus single HPV type infections if they were older, or reported consistent condom use in the previous 6 months (Table 4.2). The strongest positive correlate of multiple HPV

infections was bathing less often then daily (adjusted OR: 2.1(1.0, 4.4) vs. daily bathing). In contrast, men who reported always using a condom in the previous 6 months had a 30% lower odds of multiple HPV infections (adjusted OR: 0.7 (0.5, 0.9) versus inconsistent users).

Table 4.2. Correlates multiple HPV infections among 1,379 men with HPV infection at baseline

	Single HPV [N(%) or Median(IQR)]	Multiple HPV [N(%) or Median(IQR)]	uOR (95% CI)	aOR (95% CI)[a]
Age (years)	21 (19-22)	20 (19-22)	0.9 (0.9, 1.0)	0.9 (0.9, 1.0)
Recent travel to Nairobi[b]				
No	480 (81.4)	670 (85.5)	ref	ref
Yes	110 (18.6)	114 (14.5)	0.7 (0.6, 1.0)	0.7 (0.5, 1.0)
Bathing frequency				
Daily	571 (97.6)	751 (96.3)	ref	ref
Less than daily	14 (2.4)	29 (3.7)	1.6 (0.8, 3.0)	2.1 (1.0, 4.4)
Recent number sexual partners[b]				
0-1	330 (56.2)	417(53.1)	ref	ref
≥2	257 (43.8)	369 (47.0)	1.1 (0.9, 1.4)	1.1 (0.9, 1.4)
Recent condom use[b]				
Not always	410 (76.2)	590 (82.9)	ref	ref
Always	128(23.8)	122 (17.1)	0.7 (0.5, 0.9)	0.7 (0.5, 0.9)
N. gonorrhea				
No	565 (98.1)	754 (96.7)	ref	ref
Yes	11 (1.9)	26 (3.3)	1.8 (0.9, 3.6)	1.9 (0.9, 4.2)
C. trachomatis				
No	551 (95.8)	723 (92.7)	ref	ref
Yes	24 (4.2)	57 (7.3)	1.8 (1.1, 3.0)	1.7 (1.0, 2.8)

Abbreviations: IQR (inter-quartile range); uOR (unadjusted odds ratio); CI (confidence interval); aOR (adjusted odds ratio); ref (reference category for the odds ratio)
Missing data: travel to Nairobi (n=5), bathing frequency (n=14), number of partners in last 6 months (n=6), condom use in last 6 months (men reporting no sex in last 6 months included in always category; n=129), *N. gonorrhea* (n=23), *C. trachomatis* (n=24)
[a]Estimates are adjusted for all potential correlates
[b]Recent refers to 6 months prior to baseline

In the main analysis using semi-Bayesian logistic regression, there were no negative associations between the four vaccine-preventable HPV types (HPV-16, 18, 6 and 11) and the other 41 HPV types (ORs>1.0; Table 4.3). Although a few negative associations were observed in the semi-Bayes models with less precision and in the maximum likelihood models, all confidence intervals were wide and included the null value 1.0 (Appendix Table 4). There was no clear pattern of differences in HPV type associations within or across HPV clades. HPV-16 was positively associated with 17 of the 41 non-vaccine HPV types (range of adjusted ORs: 1.7 to 3.6). Men with HPV-16 infection were 3.6 times as likely to also be infected with HPV-40 compared to men without HPV-16 infection (Table 4.3; adjusted OR: 3.6(2.4, 5.2)). HPV-18 was positively associated with 33 non-vaccine types (range of adjusted ORs: 1.8 to 4.3), and had the strongest association with HPV-26 (adjusted OR: 4.3(2.1, 7.7)). For the vaccine-preventable low-risk types 6 and 11, 12 non-vaccine HPV types were positively associated with HPV-6, and 14 HPV types were positively associated with HPV-11. HPV-52 was most strongly associated with HPV-6 (adjusted OR: 3.4 (2.1, 5.3)) and HPV-45 was most strongly associated with HPV-11(adjusted OR: 2.8 (1.5, 4.9)).

Table 4.3. Estimated odds ratios between the four vaccine-preventable HPV types and all other non-vaccine HPV types among all men

	HPV-16 aOR (95% CI)[a]	HPV-18 aOR (95% CI)[a]	HPV-6 aOR (95% CI)[a]	HPV-11 aOR (95% CI)[a]
Clade A3				
HPV-61	2.2 (0.9, 4.5)	2.7 (1.1, 5.5)	2.0 (0.8, 4.0)	2.3 (0.9, 4.7)
HPV-72	2.7 (1.3, 4.8)	2.6 (1.2, 4.9)	1.8 (0.8, 3.4)	2.7 (1.2, 5.2)
HPV-81	1.7 (1.0, 2.8)	2.4 (1.2, 4.0)	2.8 (1.5, 4.7)	1.8 (0.8, 3.3)
HPV-83	1.8 (0.9, 3.0)	2.8 (1.4, 5.0)	1.9 (0.9, 3.5)	2.6 (1.2, 4.8)
HPV-84	1.8 (0.8, 3.4)	2.2 (1.0, 4.3)	1.8 (0.8, 3.5)	2.1 (0.9, 4.3)
HPV-89	1.7 (1.0, 2.9)	2.5 (1.3, 4.2)	1.5 (0.7, 2.8)	1.9 (0.9, 3.5)
Clade A5				
HPV-26	1.7 (0.8, 3.1)	4.3 (2.1, 7.7)	1.7 (0.8, 3.3)	2.6 (1.1, 5.1)
HPV-51	2.2 (1.4, 3.4)	1.8 (1.0, 3.1)	2.1 (1.1, 3.6)	1.8 (0.9, 3.2)
HPV-69	2.5 (1.4, 4.2)	2.8 (1.4, 5.0)	1.9 (0.9, 3.6)	1.8 (0.8, 3.4)
HPV-82	1.5 (0.7, 2.7)	2.3 (1.0, 4.3)	1.8 (0.8, 3.5)	2.7 (1.2, 5.2)
Clade A6				
HPV-53	2.6 (1.3, 4.6)	2.3 (1.0, 4.2)	1.8 (0.8, 3.4)	2.3 (1.0, 4.5)
HPV-56	2.1 (1.4, 3.0)	1.9 (1.1, 2.9)	2.9 (1.8, 4.3)	2.4 (1.3, 3.9)
HPV-66	2.0 (1.3, 3.0)	2.3 (1.3, 3.5)	1.8 (1.0, 2.9)	1.7 (0.8, 2.9)
Clade A7				
HPV-39	2.1 (1.2, 3.4)	2.2 (1.1, 3.9)	2.0 (1.0, 3.5)	2.4 (1.1, 4.4)
HPV-45	1.7 (1.1, 2.6)	2.9 (1.7, 4.6)	2.0 (1.1, 3.3)	2.8 (1.5, 4.9)
HPV-59	2.3 (1.5, 3.5)	2.9 (1.7, 4.6)	1.7 (0.9, 2.9)	2.4 (1.2, 4.2)
HPV-68	1.3 (0.7, 2.2)	2.1 (1.0, 3.9)	1.6 (0.7, 2.9)	2.1 (0.9, 4.0)
HPV-70	1.6 (0.9, 2.6)	1.7 (0.9, 3.0)	1.3 (0.6, 2.4)	2.0 (1.0, 3.8)
Clade A9				
HPV-31	2.9 (1.9, 4.2)	2.8 (1.6, 4.4)	1.6 (0.8, 2.7)	1.8 (0.9, 3.2)
HPV-33	1.9 (1.0, 3.1)	2.6 (1.3, 4.5)	1.4 (0.7, 2.6)	2.5 (1.1, 4.6)
HPV-35	2.5 (1.6, 3.7)	1.9 (1.1, 3.1)	2.4 (1.4, 3.9)	2.0 (1.0, 3.4)
HPV-52	1.7 (1.1, 2.5)	2.3 (1.3, 3.6)	3.4 (2.1, 5.3)	1.9 (1.0, 3.3)
HPV-58	2.6 (1.7, 3.9)	2.3 (1.3, 3.6)	1.7 (0.9, 2.8)	1.9 (0.9, 3.2)
Clade A10				
HPV-44	2.1 (0.9, 4.2)	2.7 (1.1, 5.5)	2.0 (0.8, 4.0)	2.3 (0.9, 4.6)
HPV-55	1.5 (0.8, 2.6)	2.0 (1.0, 3.5)	2.6 (1.3, 4.5)	2.6 (1.2, 4.8)
Clade A11				
HPV-34	2.0 (0.9, 4.0)	3.1 (1.3, 6.3)	2.0 (0.8, 4.1)	2.3 (0.9, 4.6)
HPV-64	2.1 (0.9, 4.2)	2.7 (1.1, 5.5)	2.0 (0.8, 4.0)	2.3 (0.9, 4.6)
HPV-73	2.2 (1.2, 3.5)	3.0 (1.6, 5.1)	2.0 (1.0, 3.6)	2.3 (1.1, 4.2)
Other				
HPV-30	1.7 (0.9, 2.9)	3.1 (1.5, 5.3)	1.7 (0.8, 3.1)	2.3 (1.0, 4.3)

HPV-32	1.7 (0.9, 2.9)	2.3 (1.1, 4.0)	2.4 (1.2, 4.3)	2.3 (1.0, 4.3)
HPV-40	3.6 (2.4, 5.2)	2.2 (1.3, 3.5)	2.0 (1.1, 3.3)	2.4 (1.2, 4.2)
HPV-42	2.1 (1.4, 3.0)	2.3 (1.3, 3.6)	2.0 (1.2, 3.2)	2.6 (1.4, 4.4)
HPV-43	1.7 (1.1, 2.6)	2.3 (1.3, 3.7)	3.0 (1.7, 4.8)	2.0 (1.0, 3.6)
HPV-54	1.4 (0.7, 2.6)	2.6 (1.2, 4.7)	1.8 (0.8, 3.4)	2.0 (0.9, 3.9)
HPV-57	2.2 (0.9, 4.5)	2.7 (1.1, 5.5)	2.0 (0.8, 4.0)	2.3 (0.9, 4.7)
HPV-67	2.4 (1.6, 3.4)	1.8 (1.1, 2.9)	2.6 (1.5, 4.1)	2.0 (1.0, 3.5)
HPV-71	2.2 (0.9, 4.5)	2.7 (1.1, 5.5)	2.0 (0.8, 4.0)	2.3 (0.9, 4.7)
HPV-85	2.0 (0.9, 3.8)	2.8 (1.2, 5.6)	2.1 (0.9, 4.3)	2.2 (0.9, 4.5)
HPV-86	1.8 (0.9, 3.4)	3.2 (1.5, 6.1)	1.7 (0.7, 3.2)	2.4 (1.0, 4.7)
HPV-JC9710	1.8 (1.1, 2.6)	2.8 (1.7, 4.3)	1.5 (0.8, 2.5)	2.1 (1.1, 3.7)
HPV-X	1.2 (0.8, 1.8)	1.7 (1.0, 2.7)	1.7 (1.0, 2.7)	1.3 (0.6, 2.3)

Abbreviations: aOR (adjusted odds ratio); CI (confidence interval)
[a]Odds ratios for pair-wise associations are adjusted for age, travel to Nairobi (in 6 months), bathing frequency, number of sexual partner (in 6 months), consistent condom use (in 6 months), and current Gonorrhea and *C. trachomatis* infection

Results from models including only HPV16 and HPV18 as the vaccine preventable types were almost identical to the four vaccine type models (Appendix Table 5). Additional positive associations between low-risk types HPV-6 and HPV-11 with HPV-16 and HPV-18 were observed. When analyses were restricted to HPV-positive men, point estimates tended to be closer to the null, and HPV type associations appeared not to differ within or across HPV clades (Table 4.4). HPV-40 (adjusted OR: 2.1 (1.4, 2.9)) and HPV-26 (adjusted OR: 3.4 (1.7, 5.8) remained the HPV types most strongly associated with HPV-16 and HPV-18, respectively.

Table 4.4. Estimated odds ratios between the four vaccine-preventable HPV types and all other non-vaccine HPV types among men with at least one HPV infection

	HPV-16 aOR (95% CI)[a]	HPV-18 aOR (95% CI)[a]	HPV-6 aOR (95% CI)[a]	HPV-11 aOR (95% CI)[a]
Clade A3				
HPV-61	2.1 (0.9, 4.4)	2.8 (1.1, 5.8)	2.1 (0.8, 4.2)	2.3 (1.0, 4.8)
HPV-72	2.1 (1.0, 3.6)	2.3 (1.1, 4.3)	1.6 (0.7, 3.1)	2.4 (1.1, 4.7)
HPV-81	1.1 (0.7, 1.8)	1.7 (0.9, 2.8)	2.0 (1.1, 3.3)	1.5 (0.7, 2.6)
HPV-83	1.2 (0.7, 2.0)	2.1 (1.1, 3.7)	1.6 (0.8, 2.8)	2.2 (1.1, 4.0)
HPV-84	1.5 (0.7, 2.7)	2.0 (0.9, 3.8)	1.6 (0.7, 3.0)	2.0 (0.9, 3.9)
HPV-89	1.2 (0.7, 1.8)	1.8 (1.0, 3.0)	1.2 (0.6, 2.1)	1.6 (0.8, 2.8)
Clade A5				
HPV-26	1.3 (0.7, 2.2)	3.4 (1.7, 5.8)	1.5 (0.7, 2.8)	2.3 (1.1, 4.4)
HPV-51	1.3 (0.8, 2.0)	1.3 (0.7, 2.1)	1.5 (0.8, 2.4)	1.4 (0.7, 2.4)
HPV-69	1.8 (1.0, 2.8)	2.2 (1.1, 3.7)	1.6 (0.8, 2.8)	1.6 (0.7, 2.9)
HPV-82	1.2 (0.6, 2.2)	1.9 (0.9, 3.6)	1.6 (0.7, 3.0)	2.4 (1.1, 4.6)
Clade A6				
HPV-53	1.9 (1.0, 3.4)	1.9 (0.9, 3.5)	1.6 (0.7, 3.0)	2.1 (1.0, 4.0)
HPV-56	1.2 (0.8, 1.6)	1.2 (0.7, 1.8)	1.8 (1.1, 2.6)	1.7 (0.9, 2.7)
HPV-66	1.1 (0.8, 1.6)	1.5 (0.9, 2.2)	1.2 (0.7, 1.8)	1.2 (0.6, 2.1)
Clade A7				
HPV-39	1.4 (0.8, 2.2)	1.7 (0.9, 2.9)	1.5 (0.8, 2.6)	2.0 (1.0, 3.6)
HPV-45	1.1 (0.7, 1.6)	1.9 (1.1, 3.0)	1.4 (0.8, 2.2)	2.1 (1.1, 3.5)
HPV-59	1.4 (0.9, 2.1)	2.0 (1.2, 3.1)	1.2 (0.7, 2.0)	1.8 (0.9, 3.1)
HPV-68	0.9 (0.5, 1.6)	1.7 (0.8, 2.9)	1.3 (0.6, 2.3)	1.8 (0.8, 3.2)
HPV-70	1.0 (0.6, 1.6)	1.3 (0.7, 2.2)	1.0 (0.5, 1.8)	1.7 (0.8, 3.0)
Clade A9				
HPV-31	1.7 (1.1, 2.4)	1.9 (1.1, 3.0)	1.1 (0.6, 1.9)	1.4 (0.7, 2.4)
HPV-33	1.3 (0.7, 2.0)	1.9 (1.0, 3.2)	1.2 (0.6, 2.1)	2.0 (1.0, 3.6)
HPV-35	1.4 (0.9, 2.1)	1.3 (0.8, 2.1)	1.6 (1.0, 2.5)	1.5 (0.8, 2.5)
HPV-52	1.0 (0.6, 1.4)	1.5 (0.9, 2.3)	2.1 (1.3, 3.2)	1.4 (0.8, 2.5)
HPV-58	1.5 (1.0, 2.2)	1.6 (0.9, 2.4)	1.2 (0.7, 1.9)	1.5 (0.7, 2.5)
Clade A10				
HPV-44	2.0 (0.8, 4.1)	2.7 (1.1, 5.5)	2.0 (0.8, 4.0)	2.3 (0.9, 4.8)
HPV-55	1.1 (0.6, 1.8)	1.5 (0.8, 2.7)	2.0 (1.0, 3.4)	2.1 (1.0, 3.9)
Clade A11				
HPV-34	1.9 (0.8, 3.9)	3.1 (1.3, 6.3)	1.9 (0.8, 3.8)	2.3 (0.9, 4.7)
HPV-64	2.0 (0.8, 4.1)	2.7 (1.1, 5.5)	2.0 (0.8, 4.0)	2.3 (0.9, 4.8)
HPV-73	1.4 (0.8, 2.3)	2.2 (1.2, 3.6)	1.6 (0.8, 2.6)	1.9 (0.9, 3.4)
Other				
HPV-30	1.2 (0.7, 2.0)	2.3 (1.2, 3.9)	1.4 (0.7, 2.4)	1.9 (0.9, 3.5)

HPV-32	1.2 (0.7, 2.0)	1.7 (0.9, 3.0)	1.9 (0.9, 3.2)	1.9 (0.9, 3.6)
HPV-40	2.1 (1.4, 2.9)	1.6 (0.9, 2.5)	1.4 (0.8, 2.3)	1.8 (1.0, 3.1)
HPV-42	1.2 (0.8, 1.7)	1.5 (0.9, 2.2)	1.3 (0.8, 2.0)	1.9 (1.0, 3.0)
HPV-43	1.0 (0.7, 1.5)	1.5 (0.9, 2.4)	2.0 (1.2, 3.0)	1.5 (0.8, 2.6)
HPV-54	1.1 (0.6, 2.0)	2.1 (1.0, 3.9)	1.5 (0.7, 2.9)	1.8 (0.8, 3.4)
HPV-57	2.1 (0.9, 4.4)	2.8 (1.1, 5.8)	2.1 (0.8, 4.2)	2.3 (1.0, 4.8)
HPV-67	1.3 (0.9, 1.9)	1.2 (0.7, 1.9)	1.7 (1.0, 2.5)	1.5 (0.8, 2.5)
HPV-71	2.1 (0.9, 4.4)	2.8 (1.1, 5.8)	2.1 (0.8, 4.2)	2.3 (1.0, 4.8)
HPV-85	1.8 (0.8, 3.4)	2.6 (1.2, 5.2)	2.0 (0.9, 4.0)	2.1 (0.9, 4.2)
HPV-86	1.5 (0.7, 2.8)	2.7 (1.3, 5.2)	1.5 (0.7, 2.9)	2.2 (1.0, 4.3)
HPV-JC9710	1.0 (0.7, 1.5)	1.8 (1.1, 2.7)	1.0 (0.6, 1.6)	1.6 (0.8, 2.6)
HPV-X	0.7 (0.4, 0.9)	1.1 (0.6, 1.7)	1.1 (0.6, 1.6)	0.9 (0.5, 1.6)

Abbreviations: aOR (adjusted odds ratio); CI (confidence interval)
[a]Odds ratios for pair-wise associations are adjusted for age, travel to Nairobi (in 6 months), bathing frequency, number of sexual partner (in 6 months), consistent condom use (in 6 months), and current Gonorrhea and *C. trachomatis* infection

Discussion

Half of all uncircumcised, HIV-negative men in this study from Kenya were infected with at least one HPV type at baseline. Nearly 30% of surveyed men had multiple penile HPV type infections, with HPV-16 and 18 accounting for over 25% of all infections. If prevalent carcinogenic HPV 16 and 18 infections were prevented by mass vaccination, there is a theoretical possibility that this could result in other HR-HPV types filling the ecological niche of HPV-16 and/or 18[64]. Since there are reported differences in genotype distribution across gender and geographical regions[12, 14, 65], the potential for HPV type replacement could differ across study populations. The present study represents the first detailed investigation of multiple HPV infections and type associations within a cohort of young men from Africa. Using a cross-sectional study design, we did not find any negative associations between vaccine-preventable types HPV 16, 18, 6 and 11 and 41 non-vaccine preventable HPV types.

All HPV genotypes were more likely to be detected as multiple rather than single type infections. Further, all associations between HPV types were positive, suggesting that men who become infected are more likely to have more than one HPV infection, possibly due to behavioral or immunological factors[35, 42, 55]. When analyses were restricted to HPV-infected men, associations between specific HPV types generally shifted closer towards 1.0, suggesting that specific HPV types are not associated with one another. We also observed a higher number of men than expected with four or more HPV type infections, suggesting that individual HPV infections were not independent from each other. This is likely due to the fact that men with four or more HPV infections reported less condom use in the last 6 months and more lifetime partners as compared to men with 1-3 HPV infections. These observations represent, to our knowledge, the first of their kind among men, and are consistent with results from studies among women [58-60]. Numerous prospective studies have found that women with HPV infection at baseline are more likely to acquire additional HPV types[35, 40, 42, 43, 55] and that acquisition of multiple HPV types occurs more often than expected[35, 41, 42]. It is likely that the observed pattern of number and types of HPV infections reflect differences in HPV persistence or acquisition in men.

Positive associations between HPV types were observed yet appeared not to be type-specific. Our results among Kenyan men are consistent with a prospective study of American female college students that found no two HPV types are more likely to be acquired together than any other HPV types[42]. Most studies that have examined HPV type associations in women have reported positive or no associations between HPV types, regardless of the pair-wise combinations, analytical methods, HPV genotyping methods, or study population[42, 59]. However, among female colposcopy clinic attendees in Italy,

where the genotype distribution likely differs compared to the general population, co-infection with species A7 and A10 and with HPV-31 and 52 occurred less often than expected[61]. A large cross-sectional study of Danish women referred for testing based on clinical suspicion of infection found that HPV-51 was negatively associated with HPV-16[60].

In this large sample of men with an HPV prevalence of 50%, we had the ability to look at type-type associations, even for rarer HPV types, using semi-Bayesian methods that incorporated a shrinkage factor. We chose Bayesian methods to account for all vaccine types and covariates while reducing spurious associations. There is, however, a trade-off between precision and bias in methods that incorporate a shrinkage factor[77]. We therefore conducted sensitivity analyses to understand the effect of our statistical model choices and to compare our results with other studies that used maximum likelihood estimation[58-60]. Different methods to analyze our data resulted in wider confidence intervals and, in some cases, non-significant ORs less than 1.0. However, all methods resulted in the same conclusions regarding the lack of evidence of negative associations between prevalent HPV type infections in men. Like previous studies[58-61], we made multiple comparisons across outcomes, which can increase the possibility of falsely concluding certain HPV types are associated. By including all four vaccine types in each of our models, the number of comparisons was reduced compared to other pair-wise analyses[58-61].

In the present study, we could only determine if the number and type of HPV infections were independent. The causes of non-independence may be differences in host susceptibility, the type-distribution of HPV genotypes in female sexual partners and circulating in the general population, or molecular interactions between HPV types that

inhibit infection with other HPV types (type competition). Although we were able to control for several measured confounders, it is likely that there is residual confounding by other unmeasured behavioral risk factors, immunological differences between men[35, 42] and HPV type exposure from female sexual partners. In populations where negative associations between HPV types are observed, molecular studies and prospective studies of couples are needed to address whether these associations are likely due to type competition.

Our study has additional limitations to consider when interpreting the findings. The prevalence and the type distribution of HPV has been shown to differ by circumcision status, thus the results from this cohort of uncircumcised men may not be generalizable to circumcised populations[34, 52]. In addition, no participants received HPV prophylactic vaccination, so we could only observe differences between individuals who were naturally HPV uninfected and infected with HPV-16, 18, 6 and 11. Given the cross-sectional design, it is also not known whether infection with specific HPV types affects HPV acquisition or HPV persistence of additional HPV types.

With the recent approval of HPV vaccination in men[66], these data fill an important gap in our knowledge on the distribution and associations between HPV types in multiple infections among men. Future prospective studies in populations pre- and post-vaccination are needed to observe the natural history of multiple infections and assess the long-term potential for HPV type replacement.

V: TYPE-SPECIFIC ASSOCIATIONS BETWEEN PREVALENT HPV INFECTIONS AND FUTURE ACQUISITION OF HIGH-RISK HPV TYPES IN MEN

Abstract

Background: Data in men are limited on type-specific associations between prevalent HPV infections and risk of acquiring other HPV types.

Methods: Using data from a randomized control trial of male circumcision in Kisumu, Kenya, adjusted hazard ratios (aHRs) were estimated for type-specific acquisition of any other HPV, high-risk (HR), and individual HPV types among uncircumcised men infected as compared to uninfected with HPV-16, 18, 31, 45, 6 or 11 at baseline.

Results: Among 1,064 uncircumcised men, 50% had penile HPV infections at baseline. Men with HPV-18, 31, 45, or 11, but not HPV-16 or 6, had higher rates of acquisition of any other HPV types and other HR-HPV types than men without these HPV types at baseline. Acquisition of individual HR-HPV types varied by baseline HPV type; however, we did not observe a pattern of HPV acquisition by degree of phylogenetic relatedness to the baseline infection. Except for HPV-39 among men with HPV-6 at baseline (aHR: 0.1 (0.0, 0.8)), there was no evidence of reduced HPV acquisition of any specific HPV type among men with HPV at baseline.

Conclusions: Post-vaccination surveillance studies are needed to definitely determine if prevention of HPV types by prophylactic vaccination will alter the distribution of HPV types in the population.

Introduction

Human papillomavirus (HPV) infection is the primary cause of cervical cancer in women[3, 86]. Other genital cancers, including vaginal, vulvar, anal, and penile carcinoma are also caused by HPV infection. Multiple HPV types have been detected in 20-73% of HPV infected males[14, 32-39] and are important for the risk of transmission to female sexual partners and development of anogenital cancers. With the recent approval of prophylactic HPV vaccination of young men[66], data are needed to understand if patterns of HPV acquisition differ among men with specific HPV type infections as compared to men without these HPV infections. The effect of current HPV infections on the acquisition of different HPV types could impact the long-term potential for HPV type replacement following population-based HPV vaccination[41, 64].

Previous studies have shown that infection with multiple HPV types occurs more often than expected if the infections were independent[41, 42, 58-60]. Women with HPV infection at baseline are more likely to acquire additional HPV types than those uninfected[35, 40, 42, 43, 55]. In analyses limited to five HPV types, DNA detection of either HPV-16, 18, 31, 45 or 6 did not predict acquisition of any of the other four specific HPV types among female University students[42]. In contrast, incident infections with HPV-16 and 18 were associated with higher odds of acquiring HPV-58, but not 5 other HPV types, among cytologically normal women from Colombia[41]. In a population of cytologically normal women from the United States[43] and in the ASCUS/LSIL Triage Study[55], all HPV infections, regardless of type, were associated with higher acquisition rates of other HPV types, as compared to being uninfected, likely due to a common mode of transmission or shared risk factors.

There are differences in the natural history of type-specific HPV infections between women and men[12, 65]. However, no prospective studies in men have examined the associations between specific HPV types over time. Therefore, we aim here to compare rates of grouped (overall, high-risk) and type-specific acquisition of 14 high-risk (HR) HPV types among men DNA positive as compared to DNA negative for type-specific HPV-16, 18, 31, 45, 6 or 11 infections at baseline, using data from a longitudinal cohort of young, uncircumcised men from Kisumu, Kenya.

Methods

Study population and design

A randomized controlled trial (RCT) was conducted in Kisumu, Kenya in 2002-2006 to determine the effectiveness of male circumcision in reducing the incidence of HIV infection[67]. A cohort study on the natural history of HPV infections in men was nested in the RCT[32, 68, 69]. Briefly, eligible males were between 18-24 years old, uncircumcised, HIV seronegative, and sexually active. Of the 2,702 men screened for participation in the RCT, 2,228 were enrolled. The present study is a longitudinal analysis of uncircumcised, HIV-seronegative men who were randomized to the delayed circumcision (control) arm of the RCT (n=1,102) and had HPV DNA results at baseline and at least one follow-up visit (n=1,064).

Study visits were conducted every 6 months for 24 months of follow-up. At baseline and each biannual visit, trained male interviewers administered a standardized questionnaire on socio-demographic characteristics, sexual behavior and other medical conditions. A trained physician or clinical officer conducted a physical exam during which a urine sample

was collected for PCR detection of *C. trachomatis* (Roche Diagnostics). For HPV DNA detection, penile exfoliated cells were collected from 2 anatomical sites using prewetted Dacron swabs and stored in two separate tubes: i) the shaft and external foreskin tissue and ii) the glans, coronal sulcus and inner foreskin tissue[69]. All participants provided informed consent and all study protocols were approved by the Institutional Review Boards at each collaborating university.

Detection of HPV DNA

DNA was isolated from exfoliated penile cell samples[70, 71] and the presence of human DNA was evaluated by beta-globin-specific polymerase chain reaction (PCR). HPV DNA was assessed by GP5+/6+ PCR, followed by hybridization of PCR products using an enzyme immunoassay readout with two HPV oligoprobe cocktail probes that together detect 44 HPV types. Subsequent HPV genotyping was performed by reverse line blot hybridization of the PCR products, as described previously[70, 71]. HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 were classified as HR-HPV types, and the other 30 HPV types were categorized as low-risk (LR) HPV. HPV types within the same phylogenetic clade were considered genetically-related and HPV types across different clades were considered unrelated[87]. HPV types detected by enzyme immunoassay, but not by reverse line blot genotyping, were designated as HPV X and were not included in either the high- or low-risk categories. HPV detection was performed on the shaft and glans samples separately; we present here the pooled HPV DNA results from both anatomical sites combined.

Statistical analysis

The main exposure of interest was a baseline infection with HPV-16, 18, 31, 45, 6 or 11 as compared to men without each individual type at baseline. HPV types 16, 18, 6 and 11 were chosen as baseline types of interest due to their inclusion in current generation HPV prophylactic vaccines[7, 9]. Given the relatively high prevalence of HPV 45 in adenocarcinoma and the potential for HPV cross-protection against HPV-31[10], we also investigated associations between HPV types 31 and 45 and future HPV acquisition. The study outcomes were time to acquisition of all other HPV types except the baseline type of interest, any other HR-HPV infection, and acquisition of each one of the other 13 HR-HPV types. For example, in the analysis of HPV-16 baseline infection status, we investigated acquisition of all other HPV types except HPV-16 and acquisition of all HR-HPV types except HPV-16. For all analyses, type-specific HPV acquisition was defined as the detection of a new HPV genotype at the current visit that was not detected at any of the previous study visits. Men could be simultaneously infected with multiple HPV types at any given time-point since infection with one type was not considered a competing risk for infection with another type.

Time to first infection for each HPV type was analyzed using interval censored survival methods because the time of acquisition was not directly observed, rather acquisition events were only known to have occurred between the last HPV negative visit and the first HPV positive visit[80]. If men crossed-over to the circumcision arm during the study (N=50), they were right-censored at their last visit with HPV DNA results prior to circumcision. For each HPV type where an acquisition was not observed, the data were right-censored at the final study visit. If HPV DNA results were missing for the visit(s)

before the first HPV positive visit, the acquisition interval spanned from the last non-missing HPV negative visit to the first HPV positive visit. If a result was missing between two HPV negative visits, then the missing value was assumed to be HPV negative. When this assumption was evaluated by changing the missing values from HPV negative to HPV positive, there was a minimal effect on the aHRs for HPV acquisition (data not shown).

Unadjusted survival curves were used to graphically display the pattern of HPV acquisition, stratified by HPV-16, 18, 31, 45, 6 and 11 DNA status at baseline. The survival function, which describes the probability of not acquiring an HPV infection as a function of time, was estimated using the nonparametric maximum likelihood estimator[83, 84], which is the general form of the Kaplan-Meier estimator for interval censored data. We used the published SAS macro %ICE to estimate the survival function, which is appropriate for data that are arbitrarily censored with varying interval widths[80].

Parametric frailty survival models were used to estimate the overall association between type-specific infections at baseline and acquisition of any-HPV and HR-HPV except the baseline HPV type of interest. Parametric frailty models account for the correlation between HPV types among men with multiple infections[81] and allow for interval censored data. Survival times were assumed to follow a Weibull distribution, such that the model can be viewed as either an accelerated failure time model or a proportional hazards model[81, 82]. The models were parameterized such that an adjusted hazard ratio (aHR) less than 1.0 indicates a lower rate of HPV acquisition among men positive for a specific HPV type compared to men negative for that same type at baseline, adjusted for potential confounding variables. The adjusted median survival ratio (aMSR) compares the median time to HPV acquisition among HPV infected versus uninfected men at baseline, adjusted for potential

confounding variables. Parametric survival models without the random effects frailty term were used to estimate the aHR for the association between HPV types at baseline and acquisition of each individual HR-HPV type.

All models were adjusted for potential confounding variables that were identified in the literature and our previous analysis of multiple HPV infections in this cohort, including baseline age (centered at 20 years), number of sexual partners (in 6 months prior to baseline), consistent condom use (in 6 months prior to baseline), as well as bathing frequency and baseline *C. trachomatis* infection, which were previously shown to be associated with multiple HPV infections in this cohort (Rositch *et al* (2011); submitted to JID). Analysis and interpretation of study results were based on patterns of type-specific HPV acquisition, defined by the magnitude and precision (95% confidence interval) of the estimates, rather than on statistical significance.

Results

The median age of the 1,064 men with baseline and at least one follow-up visit was 20 years (inter-quartile range [IQR] 19-22) (Table 5.1). Most men in the study population were not married (94%), unemployed (52%), and had 11 years of education (IQR: 8-12). The median age at sexual debut was 16 years (IQR: 14-17) and over half of men reported 0-1 sexual partners in the previous 6 months (57%). Consistent condom use in the previous 6 months was reported by roughly a quarter of men (23%) and *C. trachomatis* was detected in 4% of men at baseline. Nearly 70% of men had HPV DNA results available at all 5 study visits, with a median duration of 184 days (IQR: 183-187) between visits.

Table 5.1. Baseline characteristics of the uncircumcised, HIV-negative male study population (N=1,064)

	Study population N (%) or Median (IQR)[a]
Age in years	20 (19-22)
Marital status	
Not married	1000 (94.4)
Married	59 (5.6)
Employment status	
Unemployed	554 (52.3)
Employed	506 (47.7)
Years of education	11 (8-12)
Bathing frequency	
Less than daily	21 (2.0)
Daily	1030 (98.0)
Age at sexual debut	16 (14-17)
Number of sexual partners (6 months)	
0-1 Partners	599 (56.5)
≥ 2 partners	461 (43.5)
Condom use (6 months)	
Not always	731 (77.0)
Always	218 (23.0)
C. trachomatis infection	
No	1010 (96.0)
Yes	42 (4.0)
Length of testing interval (days)	184 (183-187)
Number of visits per person	5 (4-5)

Abbreviations: N (number); % (percentage); IQR (inter-quartile range)
[a]Missing: marital status (n=5), employment status (n=4), bathing frequency (n=13), age at sexual debut (n=4), Number of partners (n=4), condom use ("always" category includes men reporting no sex in last six month; n=115), *C. trachomatis* (n=12)

At baseline, 50% of men were HPV-infected, with multiple infections (n=304; 29%) detected more often than single HPV infections (n=227; 21%; p<0.01) (Table 5.2). The prevalence of baseline types of interest ranged from 10% for HPV-16 (n=101) to 2% for HPV-11 (n=19). During follow-up, the prevalence of overall HPV ranged from 49% (n=462 at 18 months) to 41% (n=366 at 24 months) and the median number of types among men

with detectable HPV was 2 (range: 1-15). A total of 127 men (12%) had no detectable HPV at all study visits. Beta-globin was detected in 72% of all penile samples (range per visit: 63-85%). The prevalence of HPV among beta-globin positive samples was very similar to the prevalence in the entire cohort, for example, 670 beta-globin positive baseline samples had an HPV prevalence of 52% (n=347; data not shown), compared to 50% at baseline.

A total of 2,233 HPV infections were acquired over study follow-up, of which 1,108 were HR-HPV types (Table 5.3). The most commonly acquired types were HPV-16 (n=178), HPV-56 (n=118), HPV-35 (n=107), HPV-42 (n=100), HPV-6 (n=97). In parametric frailty models, the rate of acquisition of any other HPV and other HR-HPV infections were higher among men with HPV-18, 31, 45, or 11 as compared to men without that type at baseline, adjusted for age, bathing frequency, condom use, number of recent sexual partners and presence of *C. trachomatis*. Infection with HPV-16 (aHR: 1.2 (95% confidence interval [CI]: 0.9, 1.6)) or HPV-6 (aHR: 1.4 (0.9, 2.1)) was not associated with higher rates of HPV acquisition. The median time to overall HPV acquisition among men with HPV-18 (aMSR: 2.2 (1.4, 3.5)) or HPV-45 (aMSR: 2.1 (1.4, 3.1.)) was over two times that of men without these infections. The same patterns of acquisition were observed for HR-HPV; however point estimates were closer to 1.0. For the HPV types included in the HPV vaccines (Table 5.3), there was an increase in any-HPV (aHR: 1.5 (1.2, 1.9)) and HR-HPV (aHR: 1.3 (1.0, 1.8)) among men with HPV-16 and/or 18 at baseline as compared to men negative to both HPV-16 and 18. Similar aHRs were observed for men with HPV-6 and/or 11 at baseline compared to those negative for both HPV-6 and 11, and for men with HPV-16, 18, 6 and/or 11 compared to men without any HPV-16, 18, 6 and 11 infection.

Table 5.2. Prevalence of HPV infection in 1,064 uncircumcised males over 24 months of follow-up

	Visit 1 (baseline) N(%) or median (range)	Visit 2 (6 months) N(%) or median (range)	Visit 3 (12 months) N(%) or median (range)	Visit 4 (18 months) N(%) or median (range)	Visit 5 (24 months) N(%) or median (range)
HPV DNA positive	531 (49.9)	426 (45.2)	462 (48.0)	462 (49.0)	366 (40.8)
Single HPV infection	227 (21.3)	204 (21.6)	217 (22.6)	217 (23.0)	166 (18.5)
Multiple HPV infections	304 (28.6)	222 (23.6)	245 (25.4)	245 (26.0)	200 (22.3)
Number of HPV types[a]	2 (1-11)	3 (1-15)	2 (1-10)	2 (1-8)	2 (1-8)
HPV-16	101 (9.5)	87 (9.2)	74 (7.8)	55 (5.8)	72 (8.0)
HPV-18	37 (3.5)	34 (3.6)	30 (3.1)	41 (4.4)	27 (3.0)
HPV-31	40 (3.8)	24 (2.6)	27 (2.8)	19 (2.0)	18 (2.0)
HPV-45	49 (4.6)	18 (1.9)	24 (2.4)	28 (3.0)	18 (2.0)
HPV-6	35 (3.3)	32 (3.4)	36 (3.7)	50 (5.3)	31 (3.5)
HPV-11	19 (1.8)	16 (1.7)	22 (2.3)	21 (2.2)	10 (1.1)
Missing HPV results[b]	0	121	102	122	166

Abbreviations: N (number); % (percentage); HPV (human papillomavirus)

[a]Among men with detectable HPV infection

[b]Includes missed study visits and samples that were inadequate for HPV testing

Table 5.3. Associations between type-specific HPV infections at baseline and future acquisition of other HPV and HR-HPV types over 24 months of follow-up

	HPV acquisition			
	Any-HPV[a] N=2,233		HR-HPV[b] N=1,108	
Baseline HPV	aHR (95% CI)[c]	aMSR (95% CI)[c]	aHR (95% CI)[c]	aMSR (95% CI)[c]
HPV-16 (n=101)	1.2 (0.9, 1.6)	1.2 (0.9, 1.7)	1.1 (0.8, 1.6)	1.1 (0.8, 1.6)
HPV-18 (n=37)	2.1 (1.4, 3.3)	2.2 (1.4, 3.5)	1.7 (1.0, 2.7)	1.7 (1.0, 2.8)
HPV-31 (n=40)	1.5 (1.0, 2.3)	1.6 (1.0, 2.4)	1.6 (1.0, 2.5)	1.6 (1.0, 2.6)
HPV-45 (n=49)	2.0 (1.4, 2.8)	2.1 (1.4, 3.1)	1.8 (1.2, 2.7)	1.8 (1.2, 2.8)
HPV-6 (n=35)	1.4 (0.9, 2.1)	1.4 (0.9, 2.2)	1.2 (0.8, 2.0)	1.2 (0.8, 2.0)
HPV-11 (n=19)	1.8 (1.0, 3.1)	1.8 (1.0, 3.3)	1.6 (0.9, 2.9)	1.6 (0.9, 3.0)
HPV-16, 18 (n=134)[d]	1.5 (1.2, 1.9)	1.5 (1.2, 2.0)	1.3 (1.0, 1.8)	1.3 (1.0, 1.8)
HPV-6, 11 (n=53)[e]	1.5 (1.0, 2.1)	1.5 (1.0, 2.2)	1.4 (1.0, 2.1)	1.4 (1.0, 2.1)
HPV-16, 18, 6, 11 (n=172)[f]	1.5 (1.2, 1.9)	1.5 (1.2, 1.9)	1.3 (1.0, 1.7)	1.3 (1.0, 1.7)

Abbreviations: HPV (human papillomavirus); HR (high-risk); aHR (adjusted hazard ratio); CI (confidence interval)

[a]Acquisition of all other HPV type infections except exposure HPV type: number of HPV-16 acquisition events=178; HPV-18=90; HPV-31=56; HPV-45=67; HPV-6=97; HPV-11=51

[b]All other HR-HPV types except exposure HPV type: HPV-16=178; HPV-18=90; HPV-31=56; HPV-45=67; HPV-6=97; HPV-11=51

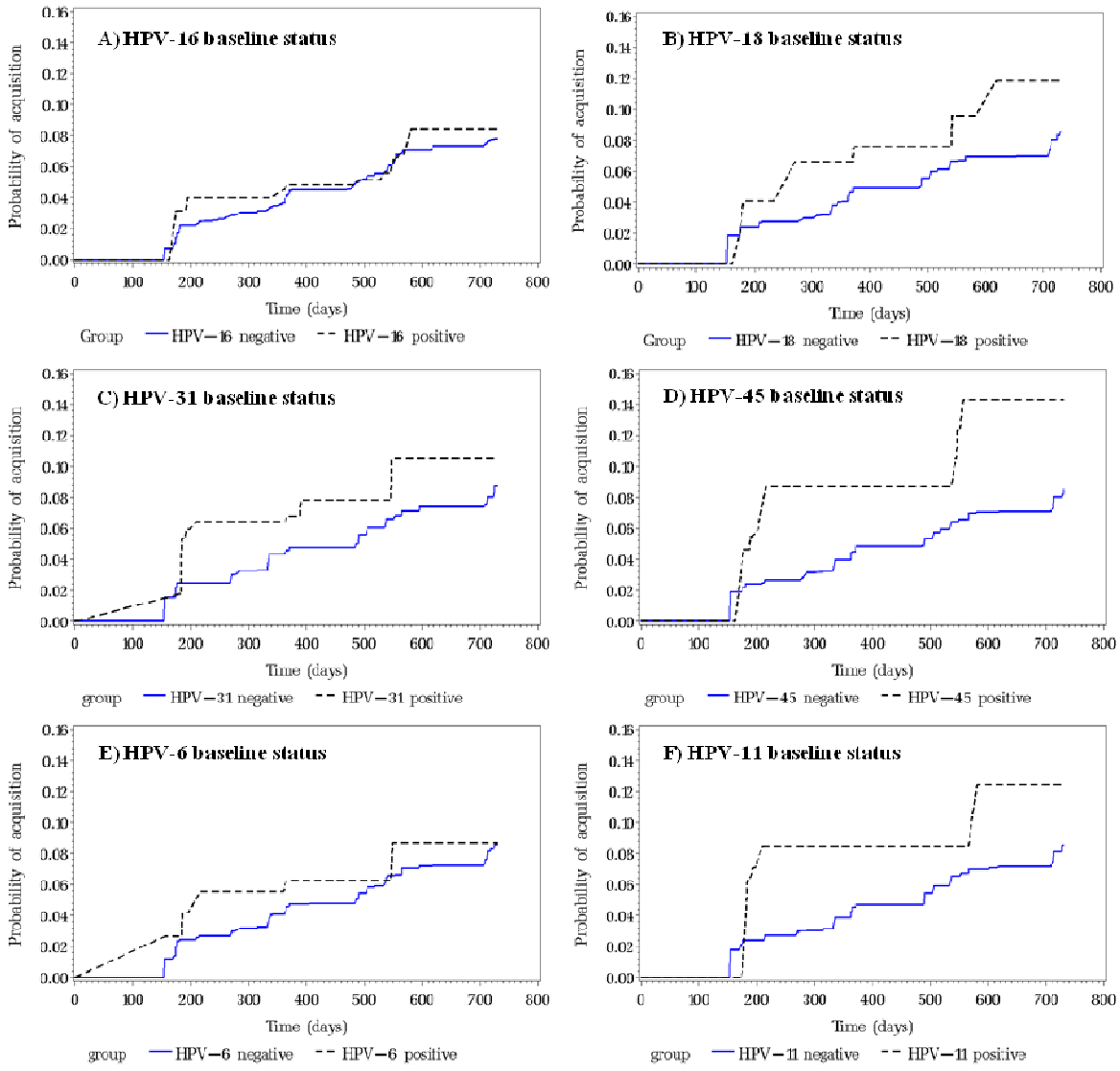
[c]Parametric frailty models adjusted for age(centered at 20 years), bathing frequency, number of sexual partners (in 6 months prior to baseline), consistent condom use (in 6 months prior to baseline), *C. trachomatis* infection

[d]Index group HPV-16 and/or 18 positive vs. referent group HPV-16 and 18 negative

[e]Index group HPV-6 and/or 11 positive vs. referent group HPV-6 and 11 negative
[f]Index group HPV-16, 18, 6, and/or 11 positive vs. referent group HPV-16, 18, 6, and 11 negative

Patterns of HR-HPV acquisition presented in the unadjusted survival curve estimates (Figure 5.1) are consistent with the results from the parametric frailty models (Table 5.3). Over study follow-up, there was no difference in HR-HPV acquisition among men infected with HPV-16 or 6 at baseline compared to uninfected men, whereas men with HPV-18 or 31 had slightly higher probabilities of HR-HPV acquisition compared to uninfected men. Baseline infection with HPV-45 and HPV-11 resulted in consistently higher probabilities of acquiring other HR-HPV types over 24 months of study follow-up.

Figure 5.1. Estimates of the cumulative probability of acquisition of other HR-HPV types over 24 months by baseline infection status.


A) HPV-16 baseline status
Probability of acquisition
Time (days)
Group HPV−16 negative HPV−16 positive
B) HPV-18 baseline status
Probability of acquisition
Time (days)
Group HPV−18 negative HPV−18 positive
C) HPV-31 baseline status
Probability of acquisition
Time (days)
group HPV−31 negative HPV−31 positive
D) HPV-45 baseline status
Probability of acquisition
Time (days)
group HPV−45 negative HPV−45 positive
E) HPV-6 baseline status
Probability of acquisition
Time (days)
group HPV−6 negative HPV−6 positive
F) HPV-11 baseline status
Probability of acquisition
Time (days)
group HPV−11 negative HPV−11 positive

Panel A) HR-HPV acquisition among men HPV-16 infected versus uninfected at baseline, B) HPV-18 infected vs. uninfected, C) HPV-31 infected vs. uninfected, D) HPV-45 infected vs. uninfected, E) HPV-6 infected vs. uninfected, and F) HPV-11 infected vs. uninfected.

To further examine the associations between baseline HPV infections and future acquisition of individual HR-HPV types, aHRs for acquisition of each of the 14 HR-HPV types were estimated (Table 5.4). Estimates of individual HR-HPV type acquisition aHRs by baseline infection status were quite variable, ranging from estimates of reduced acquisition (e.g. aHR: 0.1 (0.0, 0.8) for HPV-39 acquisition among HPV-6 DNA positive compared to negative men) to increased rates of acquisition among men with baseline infection (e.g. aHR: 4.3 (1.4, 13.1) for HPV-35 acquisition among HPV-11 DNA positive compared to negative men). Among men with HPV-16 at baseline, only the acquisition of HPV-58 (aHR: 3.9 (1.9, 8.1)) and HPV-31 (aHR: 2.3 (1.0, 5.1)) were higher than those HPV-16 uninfected at baseline. Acquisition of the 5 HR-HPV types that make up clade-A7 (HPV-18, 39, 45, 59, and 68) tended to be lower among men with HPV-16 at baseline, although the estimates were relatively imprecise. Among men with HPV-18 at baseline as compared to HPV-18 uninfected men, acquisition of each of the individual HR-HPV types appeared to be equal or slightly higher, except for HPV-31 (aHR: 0.6 (0.1, 5.5)). Similarly, for HPV-31 DNA status at baseline, aHRs for ranged from 0.4 (0.0, 3.1) for acquisition of HPV-68 to 2.3 (0.8, 6.3) for acquisition of HPV-52; however, all confidence intervals were also relative wide and included 1.0. Baseline infection with HPV-45 was associated with a higher rate of acquisition of HPV-31 (aHR: 3.1 (1.2, 8.3), HPV-56 (aHR: 1.9 (1.0, 3.6)), HPV-66 (aHR: 2.9 (1.1, 7.3)) and HPV-51 (aHR: 2.2 (1.0, 5.2)).

Baseline positivity to low-risk HPV type 6 (clade-A10) was associated with a higher rate of HPV-52 acquisition (aHR: 3.2 (1.2, 8.6))(Table 5.4). Acquisition of HPV-39 was reduced among men infected as compared to uninfected with HPV-6 at baseline

(aHR: 0.1 (0.0, 0.8)) but this estimate was based on the detection of only one incident HPV-39 infection among the 35 men with HPV-6 at baseline. Rates of acquisition of clade-A6 HPV types 56 and 66 also tended to be lower among men with HPV-6 at baseline compared to men without HPV-6. The rate of several clade-A9 types were increased among men with baseline HPV-11 (clade-A10) infection as compared to men without HPV-11, including HPV-16 (aHR: 2.7 (1.0, 7.6)), HPV-31 (aHR: 3.6 (1.0, 13.3)), HPV-35 (aHR: 4.3 (1.4, 13.1)), and HPV-58 (aHR: 2.4 (0.6, 9.9)). In contrast, several other types (HPV-39, 52, 66, 68) had point estimates less than 1.0, with relatively wide confidence intervals that included 1.0.

Comparison of rates of type-specific HPV acquisition among men with any of the four vaccine-included HPV types compared to men with none of the vaccine-included HPV types are presented in Table 5.5. The aHRs reflect the combined associations between HPV-16, 18, 6 and 11 DNA status at baseline on the risk of acquisition of each of the individual HR-HPV types over study follow-up. For example, the aHR for acquisition of HPV-58 is 3.4 (1.7, 6.9) among men with HPV-16 and/or 18 as compared to men with neither16 or 18. The results of this analysis indicate that the rate of acquisition of most non-vaccine-preventable HR-HPV types are similar or slightly higher among men infected with the HPV types included in the bivalent (HPV-16 and 18) and the quadrivalent (HPV-16, 18, 6 and 11) vaccines compared to men negative for these HPV types at baseline. However, there was a trend for lower rates of acquisition of HPV-45 and 59 among men with HPV-16 and/or HPV-18 as compared to men negative for both types, and HPV-39 and 66 among men with HPV-6 and/or HPV-11 as compared to men negative for both types.

Table 5.4. Associations between type-specific HPV infections at baseline and future acquisition of individual HR-HPV types

	Baseline HPV infection					
	HPV-16 n=101	HPV-18 n=37	HPV-31 n=40	HPV-45 n=49	HPV-6 n=35	HPV-11 n=19
HPV Acquisition	aHR(95%CI)[a]	aHR(95%CI)[a]	aHR(95%CI)[a]	aHR(95%CI)[a]	aHR(95%CI)[a]	aHR(95%CI)[a]
Clade A9						
HPV-16 (n=178)	N/A	1.4 (0.6, 3.4)	1.4 (0.6, 3.4)	1.3 (0.6, 2.7)	1.2 (0.5, 3.1)	2.7 (1.0, 7.6)
HPV-31 (n=56)	2.3 (1.0, 5.1)	0.6 (0.1, 5.5)	N/A	3.1 (1.2, 8.3)	2.0 (0.5, 7.1)	3.6 (1.0, 13.3)
HPV-33 (n=70)	1.3 (0.7, 2.3)	1.9 (0.8, 4.3)	0.9 (0.3, 2.7)	1.6 (0.8, 3.2)	0.9 (0.3, 2.9)	1.4 (0.5, 4.5)
HPV-35 (n=107)	1.0 (0.4, 2.2)	2.1 (0.7, 6.1)	1.2 (0.4, 3.9)	1.0 (0.4, 3.1)	1.2 (0.4, 3.8)	4.3 (1.4, 13.1)
HPV-52 (n=61)	0.7 (0.3, 1.9)	2.2 (0.7, 6.9)	2.3 (0.8, 6.3)	1.5 (0.6, 4.1)	3.2 (1.2, 8.6)	0.1 (0.0, 1.2)
HPV-58 (n=76)	3.9 (1.9, 8.1)	1.9 (0.6, 6.4)	2.1 (0.7, 6.4)	2.3 (0.8, 6.3)	1.5 (0.4, 5.0)	2.4 (0.6, 9.9)
Clade A7						
HPV-18 (n=90)	1.0 (0.5, 2.2)	N/A	1.2 (0.4, 3.9)	0.8 (0.2, 2.5)	1.6 (0.6, 4.4)	1.2 (0.3, 4.9)
HPV-39 (n=49)	0.7 (0.2, 2.0)	1.3 (0.3, 4.9)	1.1 (0.3, 4.3)	3.8 (1.6, 8.8)	0.1 (0.0, 0.8)	0.2 (0.0, 7.7)
HPV-45 (n=67)	0.5 (0.2, 1.5)	1.0 (0.3, 3.7)	0.5 (0.1, 2.9)	N/A	0.9 (0.2, 3.2)	1.6 (0.5, 5.9)
HPV-59 (n=56)	0.4 (0.1, 1.6)	1.5 (0.4, 5.9)	1.8 (0.6, 5.4)	1.5 (0.5, 4.6)	1.0 (0.3, 3.9)	1.2 (0.2, 8.1)
HPV-68 (n=19)	0.6 (0.1, 3.0)	1.8 (0.2, 15.2)	0.4 (0.0, 3.1)	0.4 (0.0, 3.3)	1.6 (0.2, 14.2)	0.4 (0.0, 5.2)

Clade A6						
HPV-56 (n=118)	1.0 (0.6, 1.9)	1.3 (0.5, 3.3)	2.0 (0.9, 4.0)	1.9 (1.0, 3.6)	0.3 (0.0, 2.0)	1.4 (0.5, 4.3)
HPV-66 (n=87)	0.9 (0.3, 2.3)	2.0 (0.6, 6.8)	2.3 (0.8, 7.0)	2.9 (1.1, 7.3)	0.6 (0.1, 3.5)	0.5 (0.0, 5.5)
Clade A5						
HPV-51 (n=74)	1.5 (0.7, 3.2)	2.0 (0.7, 5.8)	2.1 (0.8, 5.5)	2.2 (1.0, 5.2)	1.2 (0.4, 4.1)	1.3 (0.3, 5.5)

Abbreviations: HPV (human papillomavirus); HR (high-risk); aHR (adjusted hazard ratio); CI (confidence interval); N/A (not an applicable outcome type)

[a]Parametric survival regression models adjusted for age (centered at 20 years), bathing frequency, number of sexual partners (in 6 months), consistent condom use (in 6 months), *C. trachomatis* infection

Table 5.5. The associations between grouped HPV-16, 18, 6 and 11 infections at baseline and acquisition of individual HR-HPV types

	Baseline HPV infection		
	HPV-16, 18[a] n=134	HPV-6, 11[b] n=53	HPV-16, 18, 6,11[c] n=172
HPV acquisition	aHR(95%CI)[d]	aHR(95%CI)[d]	aHR(95%CI)[d]
Clade A9			
HPV-16 (n=178)	N/A	1.7 (0.8, 3.4)	N/A
HPV-31 (n=56)	1.9 (0.9, 4.1)	2.8 (1.0, 7.3)	2.0 (1.0, 4.0)
HPV-33 (n=70)	1.5 (0.9, 2.5)	1.1 (0.5, 2.6)	1.2 (0.7, 2.0)
HPV-35 (n=107)	1.3 (0.6, 2.5)	2.2 (0.9, 5.0)	1.3 (0.7, 2.4)
HPV-52 (n=61)	1.0 (0.5, 2.2)	1.7 (0.7, 4.4)	1.3 (0.7, 2.4)
HPV-58 (n=76)	3.4 (1.7, 6.9)	1.9 (0.7, 4.9)	3.0 (1.6, 5.8)
Clade A7			
HPV-18 (n=90)	N/A	1.5 (0.6, 3.4)	N/A
HPV-39 (n=49)	0.9 (0.4, 2.0)	0.1 (0.0, 1.3)	0.6 (0.3, 1.5)
HPV-45 (n=67)	0.6 (0.3, 1.5)	1.2 (0.5, 2.9)	0.8 (0.4, 1.5)
HPV-59 (n=56)	0.6 (0.2, 1.7)	1.1 (0.4, 3.4)	0.9 (0.4, 1.8)
HPV-68 (n=19)	0.9 (0.2, 3.6)	1.0 (0.1, 7.8)	1.0 (0.3, 3.6)
Clade A6			
HPV-56 (n=118)	1.1 (0.7, 1.9)	0.8 (0.3, 2.0)	1.0 (0.6, 1.7)
HPV-66 (n=87)	1.2 (0.5, 2.5)	0.6 (0.1, 2.4)	1.1 (0.6, 2.2)
Clade A5			
HPV-51 (n=74)	1.8 (0.9, 3.4)	1.3 (0.5, 3.3)	1.8 (1.0, 3.3)

Abbreviations: HPV (human papillomavirus); HR (high-risk); aHR (adjusted hazard ratio); CI (confidence interval); N/A (not an applicable outcome type)

[a]Vaccine-preventable high-risk types; Index group 16 and/or 18 positive vs. referent group 16 and 18 negative

[b]Vaccine-preventable low-risk types; Index group 6 and/or 11 positive vs. referent group 6 and 11 negative

[c]Index group 16, 18, 6, and/or 11 positive vs. referent group 16, 18, 6, and 11 negative

[d]Adjusted for age (centered at 20 years), bathing frequency, number of sexual partners (in 6 months), consistent condom use (in 6 months), *C. trachomatis* infection

Discussion

This study presents, to our knowledge, the first data in men on the associations between prevalent HPV infections and acquisition of other HPV types to assess the potential

for HPV type competition and replacement following wide-spread prophylactic HPV vaccination. Among 1,064 uncircumcised men from Kenya, infections with HPV-18, 31, 45, or 11 at baseline were associated with an overall higher rate of any-HPV and HR-HPV infections. Type-specific associations between baseline infections and acquisition of individual HR-HPV types varied greatly by HPV type; however, we did not observe a clear pattern in the rates of HPV acquisition by the degree of phylogenetic relatedness to the baseline infection. Except for the potentially reduced acquisition of HPV-39 among men with HPV-6 at baseline, which was based on only one incident infection among men with HPV-6 at baseline, there was no clear evidence of lower rates of acquisition of specific HR-HPV types among men with baseline HPV infection.

To better understand which HPV types were contributing to the overall positive association between prevalent infections and HPV acquisition, we analyzed acquisition of the 14 HR-HPV types in separately. There were 53 positive associations (aHRs >1.0) among the 80 type-specific comparisons, 6 aHRs were equal to 1.0, and 21 negative association between baseline HPV types and rates of type-specific acquisition (aHRs<1.). Estimates below the 1.0 were generally imprecise, with wide confidence intervals (minimum confidence limit ratio of 12.5) that included 1.0. The wide range in estimates and corresponding 95% confidence intervals for type-specific HPV acquisition (aHR range: 0.1 (0.0, 0.8) for acquisition of HPV-39 by baseline HPV-6 status, to 4.3 (1.1, 13.1) for acquisition of HPV-35 by baseline HPV-11 status) indicated that some, but not all, HR-HPV types had a higher rate of acquisition among men with baseline HPV infections. Further, we did not observe a consistent pattern of HPV acquisition by HPV type or degree of phylogenetic relatedness. There was a trend for higher acquisition of all HR-HPV types in clade-A9 among men

infected with the type HPV-45 (clade A7) as compared to uninfected men. On the other hand, acquisition of HR-HPV types in clade-A7 tended to be lower among men with unrelated type HPV-16. Among men HPV-16 positive at baseline, acquisition of two of the five phylogenetically related HR-HPV types, HPV-31 and 58, were increased as compared to men without HPV-16 at baseline. These findings are consistent with an analysis of the ASCUS/LSIL triage study, which also found a reduction in all clade-A7 types in the presence of HPV-16 infection and found HPV-31 to be increased among women with current HPV-16 infection[55].

We observed an overall trend of increased acquisition of non-vaccine-preventable HR-HPV among men with vaccine-included HPV types 16, 18, 6 and/or 11 as compared to men negative to these four types. These findings are consistent with a previous study of women from Colombia, which reported an increased risk of HPV-58 among women with HPV-16 or 18, and an increased risk of HPV-18 among women with previous HPV-6 or 11 infections[41]. It is possible that when comparing men who are negative to all four HPV types to men with at least 1-4 HPV infections, the aHRs reflect differences in HPV acquisition by risk groups rather than differences by HPV type. In fact, when we limited our analysis to men with detectable HPV during the study period (n=937), aHRs tended to be closer to 1.0, indicating smaller relative differences in rates of HPV acquisition between men with specific HPV types at baseline compared to those without each individual HPV type (Appendix Table 6).

In epidemiological studies of associations between HPV types, point estimates reflect the combined effect of factors that may increase HPV acquisition, such as unmeasured behavioral risk factors or potential biological facilitation of HPV acquisition by other HPV

types[35, 41, 42] and factors that may decrease acquisition among men with baseline HPV types, such as acquired immunity[40, 42] cross-protection[41-43] or potential HPV type competition. Because HPV acquisition is strongly related to sexual behavior, we adjusted all estimates for several potential confounding variables, including age, recent number of sexual partners, and consistent condom use. There is likely residual confounding by unmeasured sexual risk behaviors and exposure to HPV types circulating within sexual networks. Also, we examined HPV DNA positivity, not seropositivity, at baseline and acquisition of HPV types not previously detected in the study. It is possible that a previous infection with a specific HPV type could reduce the likelihood of acquiring that type over follow-up[40, 42, 88, 89] and this could potentially differ by baseline HPV status. However, men in our study population were young (median age 20 years) with relatively few lifetime partners (median 4 partners), both of which did not differ by baseline status to individual HPV types.

A strength of this prospective study in men is the use of statistical models for interval censored HPV outcomes that include a random effects frailty term to take into account the correlation among acquisition of multiple HPV types[81]. In addition, there was extensive information on potential confounding variables, including laboratory diagnosed *C. trachomatis*, which was previously shown in this population to be associated with multiple HPV infections. Despite the large sample size, a limitation of this study was the small number of events for select HPV types, which is reflected in the wide confidence intervals. In addition, the use of GP5+/6+ primers with reverse line blot hybridization likely reduces cross-hybridization between HPV types [58, 70]but might be relatively less likely to detect specific HPV types or multiple infections as compared to other detection methods[72, 73]. An additional consideration when interpreting the study results is the restriction of our

sample to young, uncircumcised men. Our findings may not be generalizable to older populations with a longer history of HPV exposure[40] or to circumcised males who may have lower rates of HPV acquisition and multiple infections[34, 52].

Using data from a large cohort of HIV-uninfected men from Kisumu, Kenya, we contribute to the previous literature in women by presenting data on the associations between baseline HPV infections and acquisition of other HPV types in men. Our young, male study population is particularly relevant given the FDA recently approved extending HPV vaccine coverage to males age 9 to 26 years[66]. Our data indicate that current infections are generally associated with a slightly higher rate of acquisition of other HPV infections. In terms of type-specific findings, there were no consistently negative associations between specific HPV types that could indicate strong potential for HPV type replacement following HPV vaccination. Ongoing vaccine surveillance studies will provide more definitive data on the potential for changes in the distribution of non-vaccine preventable HPV types following wide-spread HPV vaccination.

VI: DISCUSSION

In the 1970's, most researchers believed that a virus could not cause cervical cancer, and those who did were focused on the herpes simplex virus type 2. However, in 1983, after a decade of research, Dr. zur Hausen linked cervical cancer to novel human papillomavirus type 16, which he isolated from cervical cancer biopsies. A year later, HPV-18 was cloned from biopsy specimens. By the time Dr. zur Hausen won the Nobel Prize for Physiology and Medicine in 2008, it was well established that HPV-16 and HPV-18 cause 70% of cervical cancer cases worldwide. The discovery of HPV as the primary cause of cervical, and many other anogenital cancers, spurred one of the most productive public health research efforts in recent history that led to rapid advances in the diagnosis, treatment and prevention of cervical cancer.

In the mid-1990's HPV DNA tests were developed and incorporated into routine clinical care to identify HPV in cervical Pap smears and biopsy samples. HPV DNA tests have been shown to be more sensitive than Pap smears for the detection of cervical precancer. In 2006, the FDA approved the first prophylactic HPV vaccine, Gardasil (Merck) to prevent persistent infection with HPV-16 and 18, and low-risk HPV types 6 and 11, which are responsible for 90% of genital warts. In 2009, the bivalent Cervarix vaccine (GlaxoSmithKline) was approved for the prevention of HPV-16 and 18 and potential cross-protection against other cancer-causing HPV types 31 and 45. Wide-spread vaccination has the potential to prevent 70% of cervical cancer cases worldwide, yet there are limitations to HPV vaccination that require continued Pap smear screening and clinical research. Very little is known about the duration of protection beyond 7 years or the need for a booster in women, vaccine uptake, long-term efficacy and cost-effectiveness in males, the efficacy in

HIV-infected individuals, and possible changes in the HPV type distribution following the elimination of HPV-16 and 18 by vaccination.

Summary of findings

In the cohort of men screened for participation in the RCT, 50% of uncircumcised, HIV-negative men were HPV-infected at baseline. Nearly 30% of men had multiple penile HPV type infections, with HPV-16 and 18 accounting for over 25% of all infections. The number of HPV types detected within an individual ranged from 0 to 11 infections and all HPV genotypes were more likely to be detected as multiple rather than single type infections. We observed a higher number of men with no HPV infection and with four or more HPV type than would have been expected if each HPV infection was independent of all others. This is likely due to the fact that men with four or more HPV infections reported less condom use in the last 6 months and more lifetime partners compared to men with 1-3 HPV infections.

Using a cross-sectional study design, we did not find any negative associations between vaccine-preventable types HPV 16, 18, 6 and 11 and 41 non-vaccine preventable HPV types. Odds ratios for all pair-wise analyses ranged from 1.2 (0.8, 1.8) to 3.4 (2.1, 5.3) indicating either no association between HPV types or positive associations. HPV-16 was positively associated with 17 of the 41 non-vaccine HPV types (range of adjusted OR's: 1.7 to 3.6) and had the strongest association with low-risk HPV-40 (aOR: 3.6(2.4, 5.2)). HPV-18 had the most positive association (n=33 non-vaccine types; range of adjusted ORs: 1.8 to 4.3), and had the strongest association with low-risk HPV-26 (aOR: 4.3(2.1, 7.7)). Low risk HPV type 6 was positively associated with 12 non-vaccine HPV types and high-risk HPV-52

was most strongly associated with HPV-6 (aOR: 3.4 (2.1, 5.3)). HPV-11 was positively associated with 14 HPV types and high-risk HPV-45 was most strongly associated with HPV-11(aOR: 2.8 (1.5, 4.9)).

The findings of our prospective analysis were consistent with the main finding of the cross-sectional study: no evidence of negative associations between HPV types. The relative rates of acquisition varied by both baseline type and acquisition type but there was no evidence, with the possible exception of HPV-39 acquisition (aHR: 0.1 (0.0, 0.8)) among men with HPV-6 infection at baseline, of reduced HPV acquisition of any one of the 14 HR-HPV types among men with HPV at baseline. Infection with HPV types 18, 31, 45, or 11, but not HPV-16 or 6, at baseline were associated with an overall higher rate of any-HPV (n=2,233) and HR-HPV (n=1,108) acquisition. In addition, infection with at least one of the four vaccine-preventable HPV types compared to negative for all four HPV types was associated with an increased rate of any-HPV acquisition over 2,462 person-years of follow-up.

We did not observe a consistent pattern of association between pairs of prevalent HPV types or HPV acquisition by degree of phylogenetic relatedness. For example, there was a trend for acquisition of all HR-HPV types in clade-A9 to be *higher* among men infected with the unrelated type HPV-45 as compared to uninfected men, yet acquisition of all HR-HPV types in clade-A7 tended to be *lower* among men with unrelated type HPV-16. Interestingly, HPV-16 was not associated with an overall increase in any-HPV or HR-HPV acquisition. These results reflect the finding of the type-specific analyses, which showed that acquisition of only two HPV types was increased, while other types were not associated with baseline HPV-16 or had slightly negative associations. The only negative association

observed between all pair-wise comparisons with a confidence interval that excluded 1.0 was for the acquisition of HPV-39 among men infected with HPV-6 at baseline as compared to uninfected (aHR: 0.1 (0.0, 0.8)); however, the sample size was small, with only one HPV-39 acquisition among the 35 men with HPV-6 infection at baseline.

Study strengths and limitations

In this large sample of men, we had the ability to look at pair-wise associations, even for rare HPV types using semi-Bayesian methods. These methods allowed us to include all four vaccine types and potential confounders in each model, and reduce spurious associations between HPV types. Sensitivity analyses using maximum likelihood estimation and using semi-Bayesian methods with varying degrees of precision resulted in wider confidence intervals and, in some cases, non-significant ORs less than 1.0. Reassuring, all methods resulted in the same general conclusion regarding the lack of evidence of strong negative associations between HPV types.

The study of aim #1 raised the question as to whether the positive associations between types reflected differences in HPV acquisition or persistence. In the second study, we found evidence of increased HPV acquisition by baseline infection; a future study will examine the association between prevalent infections and HPV persistence and clearance. Like paper one, a strength of the study was the choice of statistical methods, here the use of parametric frailty models that include a random effects term to account for the correlation between HPV types among men who acquire multiple infections[81]. Unlike the cross-sectional study that made use of statistically powerful Bayesian methods, the prospective study was limited by the small number of events for specific HPV types, which is reflected in

the wide confidence intervals. In addition, the use of GP5+/6+ primers with reverse line blot hybridization likely reduces cross-hybridization between HPV types[58, 70]. On the other hand, this HPV DNA detection method may be relatively less likely to detect specific HPV types or multiple infections as compared to other methods[72, 73].

As discussed previously, the findings in these studies may not be generalizable to older men, who have longer histories of HPV exposure, or to circumcised men, who may have a reduced likelihood of HPV acquisition[40] and multiple infections[34, 52]. In addition, no participants received the HPV prophylactic vaccine, so we could only observe relative differences in HPV type associations and HPV acquisition among individuals who were naturally HPV infected and uninfected with vaccine-relevant HPV types at baseline.

Interpretation and public health significance

In epidemiological studies on HPV type associations and potential type competition, point estimates reflect differences in host susceptibility, sexual behavior, HPV type exposure from female sexual partners, and potential molecular interactions between HPV types that inhibit infection (type competition) or facilitate infection with other HPV types. Thus, in the present study, we assessed the overall association between HPV types, after controlling for several behavioral confounding variables. However, it is likely that there is residual confounding by other unmeasured behavioral risk factors and biological factors, both of which can be associated with an increase or decrease of specific HPV types in the presence of other HPV infections. The distribution of individual factors and the overall balance of positive and negative associations likely differ across study populations and analytical

adjustment for confounding, which could explain the variability in results across previous study populations.

In this population of young, uncircumcised men, current infection with vaccine-relevant HPV types was generally associated with a slightly higher rate of future acquisition of any-other HPV and HR-HPV types, after adjustment for age and other sexual behaviors. In light of the caveats above, these data are reassuring as there were no consistently negative associations between HPV types. Although we can in no way conclude that competition between HPV types does not exist, our data suggestion that at a minimum, factors that create positive associations between HPV types seem to outweigh or overwhelm the factors that may make infection with certain HPV types less likely in the presence of other HPV types. On a population level, where sexual behavior and immunological differences and potential facilitation of other infections do exist, these findings are reassuring. Just as this research does not provide definitive evidence against HPV type competition, rather just insight into the clustering patterns of HPV genotypes over time in our study population, the findings highlight other important questions regarding the role of acquired immunity, cross-protection, and infection reactivation on the long-term efficacy of the HPV vaccination.

Future research

The findings of our studies indicate that the positive associations between HPV types may be driven by increased HPV acquisition among men with prevalent HPV infections. However, the role of current HPV infections on the persistence of other types is not known. Many of the previous studies in women report increased HPV acquisition but not increased persistence by baseline infection status. However, one study in men found multiple HPV

infections to be associated with both increased HPV acquisition and increased persistence[35]. There are many issues specific to the analysis of HPV persistence, such as specifying whether the outcome HPV type is a different baseline infection or a newly acquire type, since HPV persistence is shown to differ between prevalent and incident infections[44]. In addition, a study recently showed the multiple clearance events within an individual are highly correlated[81]; thus, statistical methods need to appropriately account for the lack of independence between HPV infections. Therefore, to extend the findings of the current study and better understand the natural history of multiple HPV infections in men, the next step will be to carry-out the analysis of HPV persistence among men with vaccine-relevant HPV types compared to men without these type infections at baseline. We plan to do this complementary study using similar methods to paper two, including parametric frailty models, which account for the correlation between multiple HPV types.

With the recent approval of HPV vaccination in men[66], these data fill an important gap in our knowledge on the distribution and associations between HPV types in multiple infections among men. However, in the epidemiological current studies, we are not able to make inferences regarding the biological potential for HPV type competition. In populations where negative associations between HPV types are observed, molecular studies and prospective studies of couples are needed to address whether these associations are likely due to biological competition between HPV types. In addition, future surveillance studies in pre- and post-vaccinated populations are needed to monitor for changes in the HPV type distribution and assess the long-term potential for HPV type replacement at the population-level.

Appendix Document 1

PCR amplification protocol for HPV DNA

PCR amplification with GP5+ and biotinylated GP6+ primers, after a 4 minute denaturation step of 94˚C, 40 cycles total. Each cycle includes:

1. denaturation step at 94˚C for 20 sec
2. annealing step at 38˚C for 30 sec
3. elongation step at 71˚C for 80 seconds (84), where the final elongation step will be prolonged for a further 4 minutes

Each PCR run of 86 samples will include 2 negative sample preparation controls, 2 PCR negative controls, and a 100 pg to 10 ng dilution series of DNA of the HPV 16 containing cervical cancer cell line SiHa (containing 1 to 2 copies of HPV 16 per cell), the latter serving as positive controls.

HPV specific PCR products will be detected by hybridisation in an EIA format with two pairs of cocktail probes (i.e. high-risk HPV and low-risk HPV), together representing the 36 HPV types (84). Apart from the PCR products of samples and positive and negative PCR controls, each EIA run containing 86 samples will include 2 EIA positive controls: biotinylated PCR products derived from cloned HPV 6 (for low-risk HPV cocktail probe) and cloned HPV 16 DNA (for high-risk HPV cocktail probe) plasmids, respectively, generating an optical density (OD) value at 405nm of about 0.6 ± 0.2 after overnight substrate incubation in the EIA procedure(84). These EIA positive controls provide information about possible failure of the capturing, denaturation, hybridisation and staining steps used EIA procedures.

A GP5+/6+ PCR run will be considered valid when (a) all negative controls give OD_{405nm} values < 0.1, (b) the lowest concentration of positive PCR controls (i.e. SiHa-100 pg) gives an OD_{405nm} value near or higher than the cut-off value, and the SiHa-1 ng control a value that is clearly higher than the cut-off when using the high-risk HPV cocktail probe, and (c) the EIA positive controls give an OD_{405nm} value of >0.5. If (c) is invalid all PCR products of that particular EIA run will be subjected to an additional EIA round with new reagents. If (c) is valid but (a) and/or (b) not, the whole PCR procedure will be repeated with new PCR reagents. The following equation will be used to determine the EIA cut-off value: cut-off = 3 times the mean OD_{405nm} value of the 4 negative controls. Samples with an OD_{405nm} equal to or greater than the cut-off are considered GP5+/6+ PCR positive.

Appendix Document 2

SAS code for semi-Bayes logistic regression

```
%macro SB_IND_models (out, out2, TYPE, B1MU=, B1TAU=, B2MU=, B2TAU=, B3MU=, B3TAU=, B4MU=, B4TAU=);
ods graphics on;
proc mcmc data=HPV_ONLY
        nbi=2000 nthin=10 ntu=2000 nmc=80000
        diag=(mcse ess) outpost=&out  seed=24610;
ods select PostSummaries PostIntervals mcse ess TADpanel;

ARRAY beta[*] beta0-beta4;
ARRAY MU[*] MU1-MU4;
parms beta0-beta4 = 0;
parms MU1-MU4= 0;
prior B1MU ~normal(0,var=0.5);
prior B2MU ~normal(0,var=0.5);
prior B3MU ~normal(0,var=0.5);
prior B4MU ~normal(0,var=0.5);
prior beta0 ~ normal(0, var=1e6);
prior beta1 ~ normal(&B1MU, var =&B1TAU);
prior beta2 ~ normal(&B2MU, var =&B2TAU);
prior beta3 ~ normal(&B3MU, var =&B3TAU);
prior beta4 ~ normal(&B4MU, var =&B4TAU);

p = logistic(beta0+BETA1*OHPV6+BETA2*OHPV11+BETA3*OHPV16+BETA4*OHPV18);
model &TYPE ~ binomial(n=1,p);
run;

data &out2;
set &out;
drop LogPrior LogLike LogPost;
ARRAY beta[4] beta1-beta4;
ARRAY betaOR[4] betaOR1-betaOR4;
do i=1 to 4;
betaOR[i]=exp(beta[i]) ;
end; output;
run;

proc univariate data=&out2;
var betaOR1-betaOR4;
output out=&TYPE mean=betaOR1-betaOR4 pctlpts=2.5 97.5 pctlpre=betaOR1-betaOR4 pctlname=LCL UCL;
run;

proc print data=Pctls; run;

proc freq;
table   betaOR1*betaOR1LCL*betaOR1UCL
        betaOR2*betaOR2LCL*betaOR2UCL
        betaOR3*betaOR3LCL*betaOR3UCL
        betaOR4*betaOR4LCL*betaOR4UCL/list nocum nofreq nopercent; run;
ods graphics off;
%mend SB_IND_models;

*SB WITH INDIVIDUAL PRIORS MU=adjusted average, TAU2=0.169(5x);
%SB_IND_models (
out=HPV26, out2=postHPV26, TYPE=OHPV26, B1MU=0.535, B1TAU=0.169, B2MU=0.637, B2TAU=0.169,
B3MU=0.584, B3TAU=0.169, B4MU=1.116, B4TAU=0.169);
```

Appdendix Table 1

Distribution of HPV genotypes among men with HPV infection at baseline (N=1,379)

	Glans			Shaft			Combined		
	Single	Multiple	*p-value*[c]	Single	Multiple	*p-value*[c]	Single	Multiple	*p-value*[c]
Any HPV[a]	576 (45.9)	679 (54.1)	0.004	322 (62.5)	193 (37.5)	<0.001	592 (42.9)	787 (57.1)	<0.001
Typed infections[b]	495 (42.6)	668 (57.4)	<0.001	278 (60.0)	185 (40.0)	<0.001	507 (39.7)	769 (60.3)	<0.001
LR-HPV, exc. HR	220 (68.5)	101 (31.5)	<0.001	106 (81.5)	24 (18.5)	<0.001	211 (65.3)	112 (34.7)	<0.001
HR-HPV, inc. LR	275 (32.7)	567 (67.3)	<0.001	172 (51.7)	161 (48.7)	0.38	296 (31.1)	657(68.9)	<0.001
HR-HPV									
HPV-16	58 (28.6)	145 (71.4)	<0.001	52 (53.01)	46 (46.9)	0.04	67 (25.5)	196 (74.5)	<0.001
HPV-18	20 (20.4)	78 (79.6)	<0.001	13 (36.1)	23 (63.9)	<0.001	25 (21.6)	91 (78.4)	<0.001
HPV-31	14 (16.7)	70 (83.3)	<0.001	16 (39.0)	25 (61.0)	0.001	20 (18.4)	89 (81.6)	<0.001
HPV-33	5 (11.9)	37 (88.1)	<0.001	1 (8.3)	11 (91.7)	<0.001	4 (8.2)	45 (91.8)	<0.001
HPV-35	25 (23.2)	83 (76.9)	<0.001	14 (53.9)	12 (46.2)	0.34	27 (23.5)	88 (76.5)	<0.001
HPV-39	13 (31.0)	29 (69.0)	0.05	3 (27.3)	8 (72.7)	0.02	11 (21.6)	40 (78.4)	0.002
HPV-45	19 (23.8)	61 (76.3)	<0.001	10 (27.0)	27 (73.0)	<0.001	17 (16.8)	84 (83.2)	<0.001
HPV-51	13 (17.3)	62 (82.7)	<0.001	7 (25.0)	21 (75.0)	<0.001	13 (14.6)	76 (85.4)	<0.001
HPV-52	9 (7.9)	105 (92.1)	<0.001	3 (16.7)	15 (83.3)	<0.001	10 (7.9)	116 (92.1)	<0.001
HPV-56	27 (19.0)	115 (81.0)	<0.001	22 (39.3)	34 (60.7)	<0.001	29 (17.7)	135 (82.3)	<0.001
HPV-58	20 (21.1)	75 (78.9)	<0.001	2 (10.0)	18 (90.0)	<0.001	18 (17.7)	84 (82.4)	<0.001
HPV-59	19 (21.8)	68 (78.2)	<0.001	12 (40.0)	18 (60.0)	0.008	19 (19.6)	78 (80.4)	<0.001
HPV-66	25 (21.2)	92 (78.8)	<0.001	12 (29.3)	29 (70.7)	<0.001	22 (16.1)	115 (83.9)	<0.001
HPV-68	4 (11.4)	31 (88.6)	<0.001	1 (14.3)	6 (85.7)	0.01	4 (10.5)	34 (89.5)	<0.001
LR-HPV									
HPV-6	23 (23.7)	74 (76.3)	<0.001	8 (28.6)	20 (71.4)	<0.001	22 (20.4)	86 (79.6)	<0.001
HPV-11	13 (26.0)	37 (74.0)	0.004	11 (64.7)	6 (35.3)	0.86	13 (23.2)	43 (76.8)	0.002
HPV-26	4 (16.7)	20 (83.3)	0.003	2 (25.0)	6 (75.0)	0.06	5 (20.0)	20 (80.0)	0.02
HPV-30	7 (19.4)	29 (80.6)	0.001	2 (20.0)	8 (80.0)	0.007	6 (14.3)	36 (85.7)	<0.001
HPV-32	2 (5.1)	37 (94.9)	<0.001	2 (33.3)	4 (66.7)	0.20	2 (4.8)	40 (95.2)	<0.001
HPV-34	0 (0)	1 (100.0)	1.00	0 (0)	1 (100.0)	0.37	0 (0)	2 (100.0)	0.51

HPV-40	14 (13.9)	87 (86.1)	<0.001	7 (28.0)	18 (72.0)	<0.001	10 (9.0)	101 (91.0)	<0.001
HPV-42	24 (19.4)	100 (80.7)	<0.001	7 (18.4)	31 (81.6)	<0.001	20 (14.3)	120 (85.7)	<0.001
HPV-43	15 (14.6)	88 (85.4)	<0.001	13 (39.4)	20 (60.6)	0.004	17 (14.9)	97 (85.1)	<0.001
HPV-44	0 (0)	0 (0)	---	1 (100.0)	0 (0)	1.00	1 (100.0)	0 (0)	0.43
HPV-53	3 (16.7)	15 (83.3)	0.02	0 (0)	7 (100.0)	<0.001	1 (4.8)	20 (95.2)	<0.001
HPV-54	4 (18.2)	18 (81.8)	0.009	0 (0)	1 (100.0)	0.37	4 (18.2)	18 (81.8)	0.02
HPV-55	5 (11.6)	38 (88.4)	<0.001	2 (33.3)	4 (66.7)	0.20	6 (13.3)	39 (86.7)	<0.001
HPV-57	0 (0)	0 (0)	---	0 (0)	0 (0)	---	0 (0)	0 (0)	---
HPV-61	0 (0)	0 (0)	---	0 (0)	0 (0)	---	0 (0)	0 (0)	---
HPV-64	0 (0)	1 (100.0)	1.00	0 (0)	0 (0)	---	0 (0)	1 (100.0)	1.00
HPV-67	23 (18.9)	99 (81.2)	<0.001	11 (29.7)	26 (70.3)	<0.001	22 (15.8)	117 (84.2)	<0.001
HPV-69	7 (21.9)	25 (78.1)	0.006	2 (18.2)	9 (81.8)	0.003	5 (12.8)	34 (87.2)	<0.001
HPV-70	18 (32.1)	38 (67.9)	0.03	3 (21.4)	11 (78.6)	0.003	18 (29.0)	44 (71.0)	0.02
HPV-71	0 (0)	0 (0)	---	0 (0)	0 (0)	---	0 (0)	0 (0)	---
HPV-72	2 (11.8)	15 (88.2)	0.005	2 (22.2)	7 (77.8)	0.02	2 (10.5)	17 (89.5)	0.004
HPV-73	5 (9.1)	50 (90.9)	<0.001	1 (20.0)	4 (80.0)	0.07	4 (6.9)	54 (93.1)	<0.001
HPV-81	10 (17.0)	49 (83.1)	<0.001	4 (20.0)	16 (80.0)	<0.001	10 (14.9)	54 (85.1)	<0.001
HPV-82	4 (22.2)	14 (77.8)	0.06	1 (50.0)	1 (50.0)	1.00	5 (25.0)	15 (75.0)	0.12
HPV-83	4 (10.8)	33 (89.2)	<0.001	2 (18.2)	9 (81.8)	0.003	4 (9.8)	37 (90.2)	<0.001
HPV-84	2 (16.7)	10 (83.3)	0.05	0 (0)	0 (0)	---	1 (8.3)	11 (91.7)	0.02
HPV-85	2 (28.6)	5 (71.4)	0.46	0 (0)	0 (0)	---	2 (28.6)	5 (71.4)	0.71
HPV-86	3 (25.0)	9 (75.0)	0.24	1 (33.3)	2 (66.7)	0.56	4 (26.7)	11 (73.3)	0.30
HPV-89	7 (13.2)	46 (86.8)	<0.001	0 (0)	8 (100.0)	<0.001	6 (11.1)	48 (88.9)	<0.001
HPV-JC9710	12 (10.5)	102 (89.5)	<0.001	20 (48.8)	21 (51.2)	0.06	13 (10.3)	113 (89.7)	<0.001
Untyped HPV	92 (78.0)	26 (22.0)	<0.001	52 (81.3)	12 (18.8)	0.001	103 (62.8)	61 (37.2)	<0.001

[a] Any HPV includes untypes HPV infections (HPV-X)

[b]Typed HPV infection excludes HPV-X

[c]If there were 5 observations or less, the Fisher's exact two-sided p-value is reported

Abbreviations: HR-HPV (high-risk human papillomavirus); LR-HPV (low-risk human papillomavirus)

Appendix Table 2

Parametric survival models with and without frailty term and covariates

	Any-HPV		HR-HPV	
	HR (95%CI)	MSR (95%CI)	HR (95%CI)	MSR (95%CI)
No frailty term, unadjusted				
HPV-16	1.2 (1.1, 1.4)	1.3 (1.1, 1.5)	1.2 (1.0, 1.5)	1.2 (1.0, 1.5)
HPV-18	1.8 (1.4, 2.2)	1.8 (1.5, 2.3)	1.6 (1.2, 2.2)	1.6 (1.2, 2.3)
HPV-31	1.4 (1.1, 1.7)	1.4 (1.1, 1.8)	1.5 (1.1, 2.0)	1.5 (1.1, 2.1)
HPV-45	1.9 (1.6, 2.2)	2.0 (1.6, 2.4)	1.8 (1.4, 2.4)	1.9 (1.4, 2.5)
HPV-6	1.6 (1.2, 1.9)	1.6 (1.3, 2.1)	1.3 (0.9, 1.8)	1.3 (0.9, 1.8)
HPV-11	1.7 (1.3, 2.2)	1.8 (1.3, 2.4)	1.7 (1.2, 2.5)	1.8 (1.2, 2.7)
No frailty, adjusted[a]				
HPV-16	1.2 (1.1, 1.4)	1.2 (1.1, 1.4)	1.2 (0.9, 1.5)	1.2 (0.9, 1.4)
HPV-18	1.8 (1.5, 2.3)	1.8 (1.5, 2.3)	1.6 (1.2, 2.2)	1.6 (1.1, 2.2)
HPV-31	1.4 (1.1, 1.8)	1.4 (1.1, 1.7)	1.6 (1.2, 2.1)	1.5 (1.1, 2.0)
HPV-45	1.9 (1.6, 2.3)	1.9 (1.6, 2.3)	1.8 (1.4, 2.3)	1.8 (1.4, 2.3)
HPV-6	1.4 (1.1, 1.7)	1.4 (1.1, 1.7)	1.2 (0.8, 1.7)	1.2 (0.8, 1.6)
HPV-11	1.7 (1.3, 2.2)	1.7 (1.3, 2.2)	1.8 (1.3, 2.6)	1.8 (1.2, 2.5)
Frailty models, Unadjusted				
HPV-16	1.2 (0.9, 1.6)	1.2 (0.9, 1.7)	1.2 (0.8, 1.6)	1.2 (0.8, 1.6)
HPV-18	2.0 (1.3, 3.0)	2.1 (1.3, 3.2)	1.6 (1.0, 2.6)	1.6 (1.0, 2.6)
HPV-31	1.5 (1.0, 2.3)	1.6 (1.0, 2.4)	1.6 (1.0, 2.4)	1.6 (1.0, 2.5)
HPV-45	2.1 (1.4, 2.9)	2.2 (1.5, 3.1)	2.0 (1.3, 2.9)	2.0 (1.3, 3.0)
HPV-6	1.5 (1.0, 2.3)	1.5 (1.0, 2.4)	1.3 (0.8, 2.1)	1.3 (0.8, 2.1)
HPV-11	1.9 (1.1, 3.3)	2.0 (1.1, 3.5)	1.7 (0.9, 3.1)	1.7 (0.9, 3.1)
Frailty models, adjusted[a]				
HPV-16	1.2 (0.9, 1.6)	1.2 (0.9, 1.7)	1.1 (0.8, 1.6)	1.1 (0.8, 1.6)
HPV-18	2.1 (1.4, 3.3)	2.2 (1.4, 3.5)	1.7 (1.0, 2.7)	1.7 (1.0, 2.8)
HPV-31	1.5 (1.0, 2.3)	1.6 (1.0, 2.4)	1.6 (1.0, 2.5)	1.6 (1.0, 2.6)
HPV-45	2.0 (1.4, 2.8)	2.1 (1.4, 3.1)	1.8 (1.2, 2.7)	1.8 (1.2, 2.8)
HPV-6	1.4 (0.9, 2.1)	1.4 (0.9, 2.2)	1.2 (0.8, 2.0)	1.2 (0.8, 2.0)
HPV-11	1.8 (1.0, 3.1)	1.8 (1.0, 3.3)	1.6 (0.9, 2.9)	1.6 (0.9, 3.0)

Abbreviations: HR-HPV (high-risk HPV); HR (hazard ratio); CI (confidence interval); MSR (median survival ratio)

[a]Adjusted for age, bathing frequency, number of sexual partners (in 6 months), consistent condom use (in 6 months), *C. trachomatis* infection

Appendix Table 3

Sensitivity analysis results for any-HPV acquisition comparing methods used to code missing data for time to event analysis

	Alternate interval coding: if HPV data was missing outside of the acquisition interval, assumed missing value was HPV positive		**Original interval coding**: if HPV data was missing outside of the acquisition interval, assumed missing value was HPV negative	
	aHR (95% CI) [a,b]	aMSR (95%CI)[a,b]	aHR (95% CI) [a,b]	aMSR (95% CI) [a,b]
HPV-16	1.2 (1.0, 1.8)	1.2 (1.0, 1.7)	1.2 (0.9, 1.6)	1.2 (0.9, 1.7)
HPV-18	2.0 (1.2, 3.3)	2.0 (1.2, 3.2)	2.1 (1.4, 3.3)	2.2 (1.4, 3.5)
HPV-31	1.7 (1.0, 2.8)	1.6 (1.0, 2.6)	1.5 (1.0, 2.3)	1.6 (1.0, 2.4)
HPV-45	2.0 (1.4, 2.9)	1.9 (1.3, 3.0)	2.0 (1.4, 2.8)	2.1 (1.4, 3.1)
HPV-6	1.4 (0.9, 2.1)	1.4 (0.9, 2.1)	1.4 (0.9, 2.1)	1.4 (0.9, 2.2)
HPV-11	1.9 (1.1, 3.1)	1.9 (1.0, 3.2)	1.8 (1.0, 3.1)	1.8 (1.0, 3.3)
HPV-16, 18[c]	1.5 (1.2, 2.0)	1.5 (1.1, 1.9)	1.5 (1.2, 1.9)	1.5 (1.2, 2.0)
HPV-6, 11[d]	1.5 (1.0, 2.2)	1.5 (1.0, 2.3)	1.5 (1.0, 2.1)	1.5 (1.0, 2.2)
HPV-16, 18, 6, 11[e]	1.5 (1.2, 2.0)	1.5 (1.2, 1.9)	1.5 (1.2, 1.9)	1.5 (1.2, 1.9)

Abbreviations: aHR (adjusted hazard ratio); CI (confidence interval); aMSR (adjusted median survival ratio)

[a]Parametric frailty models adjusted for age (centered at 20 years), bathing frequency, number of sexual partners (in 6 months), consistent condom use (in 6 months), *C. trachomatis* infection

[b]All other HPV types except exposure HPV type

[c]Index group HPV-16 and/or 18 positive vs. referent group HPV-16 and 18 negative

[d]Index group HPV-6 and/or 11 positive vs. referent group HPV-6 and 11 negative

[e]Index group HPV-16, 18, 6, and/or 11 positive vs. referent group HPV-16, 18, 6, and 11 negative

Appendix Table 4

Sensitivity analysis comparing maximum likelihood logistic regression to semi-Bayesian logistic regression with varying estimates of precision for the prior probabilities

	HPV-16[a]				HPV-18[a]			
	MLE model	Assume 100-fold OR range	Assume 10-fold OR range	Assume 5-fold OR range	MLE model	Assume 100-fold OR range	Assume 10-fold OR range	Assume 5-fold OR range
HPV-26	0.7 (0.2, 2.7)	1.2 (0.3, 2.9)	1.5 (0.6, 3.1)	1.7 (0.8, 3.1)	10.6 (4.0, 28.0)	8.3 (2.9, 17.3)	5.6 (2.4, 11.0)	4.3 (2.1, 7.7)
HPV-30	1.2 (0.5, 3.2)	1.5 (0.6, 3.0)	1.6 (0.8, 3.0)	1.7 (0.9, 2.9)	3.7 (1.4, 9.8)	3.7 (1.3, 7.5)	3.3 (1.5, 6.2)	3.1 (1.5, 5.3)
HPV-31	2.9 (1.8, 4.8)	3.2 (2.0, 4.9)	3.0 (1.9, 4.5)	2.9 (1.9, 4.2)	2.9 (1.5, 5.7)	2.9 (1.5, 5.0)	2.9 (1.6, 4.8)	2.8 (1.6, 4.4)
HPV-32	1.3 (0.5, 3.4)	1.5 (0.6, 3.2)	1.6 (0.7, 3.0)	1.7 (0.9, 2.9)	1.3 (0.3, 5.7)	1.9 (0.5, 4.4)	2.1 (0.8, 4.2)	2.3 (1.1, 4.0)
HPV-33	1.6 (0.7, 3.6)	1.8 (0.7, 3.3)	1.8 (0.9, 3.2)	1.9 (1.0, 3.1)	2.3 (0.8, 6.7)	2.6 (0.9, 5.5)	2.6 (1.1, 4.9)	2.6 (1.3, 4.5)
HPV-34[b]	N/C	2.0 (0.2, 8.4)	2.0 (0.6, 5.3)	2.0 (0.9, 4.0)	33.4 (1.9, 577.0)	8.3 (0.7, 33.7)	3.8 (1.1, 9.5)	3.1 (1.3, 6.3)
HPV-35	2.6 (1.6, 4.3)	2.7 (1.7, 4.2)	2.6 (1.6, 3.9)	2.5 (1.6, 3.7)	1.6 (0.8, 3.5)	1.6 (0.7, 3.0)	1.8 (0.9, 3.1)	1.9 (1.1, 3.1)
HPV-39	1.9 (0.9, 4.0)	2.2 (1.0, 4.0)	2.2 (1.1, 3.8)	2.1 (1.2, 3.4)	1.8 (0.6, 5.3)	2.0 (0.6, 4.2)	2.2 (0.9, 4.2)	2.2 (1.1, 3.9)
HPV-40	3.7 (2.3, 5.9)	4.2 (2.7, 6.3)	3.9 (2.5, 5.7)	3.6 (2.4, 5.2)	1.7 (0.8, 3.7)	2.0 (0.9, 3.6)	2.1 (1.1, 3.6)	2.2 (1.3, 3.5)
HPV-42	2.2 (1.3, 3.5)	2.1 (1.3, 3.2)	2.1 (1.3, 3.1)	2.1 (1.4, 3.0)	2.1 (1.1, 4.1)	2.1 (1.1, 3.6)	2.2 (1.2, 3.7)	2.3 (1.3, 3.6)
HPV-43	1.7 (1.0, 2.9)	1.7 (0.9, 2.6)	1.7 (1.0, 2.6)	1.7 (1.1, 2.6)	2.4 (1.2, 4.9)	2.2 (1.0, 3.9)	2.2 (1.2, 3.7)	2.3 (1.3, 3.7)
HPV-44[b]	N/C	2.6 (0.2, 11.6)	2.2 (0.6, 5.6)	2.1 (0.9, 4.2)	N/C	3.8 (0.2, 18.0)	2.9 (0.8, 7.5)	2.7 (1.1, 5.5)
HPV-45	1.5 (0.8, 2.7)	1.6 (0.9, 2.7)	1.7 (1.0, 2.6)	1.7 (1.1, 2.6)	2.7 (1.3, 5.4)	3.1 (1.5, 5.4)	3.0 (1.6, 5.0)	2.9 (1.7, 4.6)
HPV-51	2.3 (1.3, 4.0)	2.4 (1.3, 3.8)	2.3 (1.3, 3.6)	2.2 (1.4, 3.4)	1.3 (0.5, 3.5)	1.4 (0.5, 2.8)	1.6 (0.7, 2.9)	1.8 (1.0, 3.1)
HPV-52	1.1 (0.6, 2.0)	1.6 (0.9, 2.5)	1.6 (1.0, 2.5)	1.7 (1.1, 2.5)	2.4 (1.2, 4.7)	2.2 (1.0, 3.8)	2.2 (1.2, 3.7)	2.3 (1.3, 3.6)
HPV-53	3.8 (1.3, 10.9)	3.5 (1.2, 7.6)	2.9 (1.2, 5.6)	2.6 (1.3, 4.6)	1.1 (0.1, 8.5)	1.5 (0.3, 4.5)	2.0 (0.7, 4.6)	2.3 (1.0, 4.2)
HPV-54	0.5 (0.1, 3.6)	0.8 (0.2, 2.2)	1.2 (0.5, 2.6)	1.4 (0.7, 2.6)	1.4 (0.2, 10.8)	2.6 (0.5, 6.7)	2.6 (0.9, 5.5)	2.6 (1.2, 4.7)
HPV-55	1.3 (0.5, 3.4)	1.2 (0.4, 2.4)	1.4 (0.6, 2.6)	1.5 (0.8, 2.6)	1.1 (0.3, 4.8)	1.3 (0.3, 3.3)	1.7 (0.7, 3.5)	2.0 (1.0, 3.5)
HPV-56	1.9 (1.2, 2.9)	2.2 (1.4, 3.2)	2.2 (1.4, 3.1)	2.1 (1.4, 3.0)	1.6 (0.8, 3.2)	1.6 (0.8, 2.8)	1.7 (0.9, 2.8)	1.9 (1.1, 2.9)
HPV-57[b]	N/C	3.9 (0.2, 19.3)	2.3 (0.6, 5.9)	2.2 (0.9, 4.5)	N/C	5.0 (0.3, 24.6)	3.0 (0.8, 8.2)	2.7 (1.1, 5.5)
HPV-58	3.2 (1.9, 5.3)	2.9 (1.7, 4.5)	2.7 (1.7, 4.2)	2.6 (1.7, 3.9)	1.4 (0.6, 3.3)	2.1 (0.9, 3.8)	2.2 (1.1, 3.7)	2.3 (1.3, 3.6)
HPV-59	2.2 (1.3, 3.8)	2.5 (1.4, 4.0)	2.4 (1.5, 3.7)	2.3 (1.5, 3.5)	3.4 (1.7, 6.5)	3.1 (1.6, 5.5)	3.0 (1.6, 5.0)	2.9 (1.7, 4.6)
HPV-61[b]	N/C	3.9 (0.2, 19.3)	2.3 (0.6, 5.9)	2.2 (0.9, 4.5)	N/C	5.0 (0.3, 24.6)	3.0 (0.8, 8.2)	2.7 (1.1, 5.5)

HPV-64[b]	N/C	2.6 (0.2, 11.6)	2.2 (0.6, 5.6)	2.1 (0.9, 4.2)	N/C	3.8 (0.2, 18.0)	2.9 (0.8, 7.5)	2.7 (1.1, 5.5)
HPV-66	1.9 (1.2, 3.1)	2.1 (1.3, 3.2)	2.1 (1.3, 3.1)	2.0 (1.3, 3.0)	2.1 (1.1, 4.0)	2.1 (1.1, 3.6)	2.2 (1.2, 3.6)	2.3 (1.3, 3.5)
HPV-67	2.2 (1.4, 3.6)	2.5 (1.6, 3.7)	2.4 (1.5, 3.6)	2.4 (1.6, 3.4)	1.6 (0.8, 3.4)	1.5 (0.7, 2.7)	1.7 (0.9, 2.8)	1.8 (1.1, 2.9)
HPV-68	0.5 (0.1, 2.2)	0.7 (0.2, 1.8)	1.1 (0.4, 2.1)	1.3 (0.7, 2.2)	1.4 (0.3, 6.2)	1.6 (0.4, 4.2)	2.0 (0.7, 4.1)	2.1 (1.0, 3.9)
HPV-69	3.0 (1.4, 6.6)	3.1 (1.3, 5.8)	2.7 (1.4, 4.8)	2.5 (1.4, 4.2)	3.4 (1.3, 9.2)	3.0 (1.0, 6.5)	2.9 (1.2, 5.5)	2.8 (1.4, 5.0)
HPV-70	1.3 (0.5, 3.0)	1.5 (0.7, 2.8)	1.5 (0.8, 2.7)	1.6 (0.9, 2.6)	0.9 (0.2, 3.9)	1.0 (0.3, 2.4)	1.5 (0.6, 2.9)	1.7 (0.9, 3.0)
HPV-71[b]	N/C	3.9 (0.2, 19.3)	2.3 (0.6, 5.9)	2.2 (0.9, 4.5)	N/C	5.0 (0.3, 24.6)	3.0 (0.8, 8.2)	2.7 (1.1, 5.5)
HPV-72	3.6 (1.2, 10.8)	3.7 (1.3, 8.3)	3.1 (1.3, 6.0)	2.7 (1.3, 4.8)	2.8 (0.6, 12.9)	2.5 (0.5, 6.6)	2.6 (0.9, 5.6)	2.6 (1.2, 4.9)
HPV-73	2.2 (1.1, 4.4)	2.2 (1.1, 3.9)	2.2 (1.2, 3.6)	2.2 (1.2, 3.5)	3.0 (1.3, 7.0)	3.4 (1.5, 6.5)	3.2 (1.6, 5.7)	3.0 (1.6, 5.1)
HPV-81	1.4 (0.7, 2.9)	1.6 (0.7, 2.8)	1.6 (0.9, 2.8)	1.7 (1.0, 2.8)	2.4 (1.0, 5.8)	2.2 (0.8, 4.3)	2.3 (1.1, 4.2)	2.4 (1.2, 4.0)
HPV-82	0.4 (0.1, 3.2)	0.9 (0.2, 2.4)	1.3 (0.5, 2.6)	1.5 (0.7, 2.7)	1.5 (0.2, 11.5)	1.8 (0.3, 5.2)	2.1 (0.7, 4.7)	2.3 (1.0, 4.3)
HPV-83	1.3 (0.5, 3.4)	1.5 (0.6, 3.1)	1.6 (0.8, 3.0)	1.8 (0.9, 3.0)	2.0 (0.6, 6.9)	3.1 (1.0, 6.7)	2.9 (1.2, 5.6)	2.8 (1.4, 5.0)
HPV-84	0.9 (0.1, 7.2)	1.3 (0.2, 3.9)	1.6 (0.6, 3.7)	1.8 (0.8, 3.4)	N/C	1.4 (0.1, 5.1)	2.0 (0.6, 4.8)	2.2 (1.0, 4.3)
HPV-85	1.2 (0.1, 10.4)	1.9 (0.3, 6.4)	2.0 (0.6, 4.8)	2.0 (0.9, 3.8)	3.4 (0.4, 30.0)	3.7 (0.5, 12.1)	3.1 (0.9, 7.2)	2.8 (1.2, 5.6)
HPV-86	0.8 (0.1, 6.2)	1.5 (0.3, 4.0)	1.8 (0.7, 3.9)	1.8 (0.9, 3.4)	5.5 (1.1, 27.0)	5.1 (1.2, 12.9)	3.8 (1.3, 8.1)	3.2 (1.5, 6.1)
HPV-89	1.8 (0.8, 4.0)	1.6 (0.7, 3.0)	1.7 (0.8, 2.9)	1.7 (1.0, 2.9)	2.6 (1.0, 6.9)	2.4 (0.9, 4.8)	2.4 (1.1, 4.5)	2.5 (1.3, 4.2)
HPV-JC9710	1.5 (0.9, 2.6)	1.7 (1.0, 2.6)	1.7 (1.0, 2.6)	1.8 (1.1, 2.6)	3.3 (1.8, 6.2)	2.9 (1.5, 4.9)	2.9 (1.6, 4.6)	2.8 (1.7, 4.3)
HPV-X	1.2 (0.7, 2.1)	1.0 (0.6, 1.6)	1.1 (0.7, 1.7)	1.2 (0.8, 1.8)	1.4 (0.7, 2.9)	1.4 (0.7, 2.5)	1.6 (0.8, 2.6)	1.7 (1.0, 2.7)

Abbreviations: N/C (sparse or no data, could not calculate estimate)

[a]Odds ratios for pair-wise associations are adjusted for age, travel to Nairobi (in 6 months), bathing frequency, number of sexual partner (in 6 months), consistent condom use (in 6 months), and current Gonorrhea and *C. trachomatis* infection

[b]HPV-57, HPV-61, HPV-71 no infections; HPV-34, HPV-64: no single infections; HPV-44 no multiple infections

	HPV-6[a]				HPV-11[a]			
	MLE model	Assume 100-fold OR range	Assume 10-fold OR range	Assume 5-fold OR range	MLE model	Assume 100-fold OR range	Assume 10-fold OR range	Assume 5-fold OR range
HPV-26	0.8 (0.1, 6.2)	1.3 (0.2, 3.9)	1.6 (0.5, 3.7)	1.7 (0.8, 3.3)	6.8 (1.4, 32.1)	3.9 (0.7, 10.5)	2.9 (0.9, 6.6)	2.6 (1.1, 5.1)
HPV-30	1.1 (0.3, 4.8)	1.4 (0.3, 3.5)	1.6 (0.6, 3.3)	1.7 (0.8, 3.1)	2.4 (0.5, 10.6)	2.5 (0.5, 6.5)	2.4 (0.8, 5.1)	2.3 (1.0, 4.3)
HPV-31	1.2 (0.5, 3.0)	1.4 (0.5, 2.6)	1.5 (0.7, 2.7)	1.6 (0.8, 2.7)	1.4 (0.4, 4.7)	1.4 (0.4, 3.2)	1.7 (0.7, 3.3)	1.8 (0.9, 3.2)
HPV-32	3.6 (1.3, 9.7)	3.1 (1.0, 6.7)	2.7 (1.1, 5.2)	2.4 (1.2, 4.3)	2.5 (0.5, 11.6)	2.4 (0.5, 6.4)	2.4 (0.8, 5.1)	2.3 (1.0, 4.3)
HPV-33	0.5 (0.1, 3.5)	0.8 (0.2, 2.2)	1.2 (0.4, 2.5)	1.4 (0.7, 2.6)	2.0 (0.5, 8.7)	3.1 (0.8, 7.4)	2.7 (1.0, 5.6)	2.5 (1.1, 4.6)
HPV-34[b]	N/C	2.5 (0.2, 10.5)	2.1 (0.5, 5.4)	2.0 (0.8, 4.1)	N/C	3.3 (0.2, 14.9)	2.5 (0.7, 6.7)	2.3 (0.9, 4.6)
HPV-35	2.4 (1.2, 4.7)	2.8 (1.4, 4.8)	2.6 (1.4, 4.4)	2.4 (1.4, 3.9)	1.2 (0.4, 4.1)	1.7 (0.5, 3.7)	1.9 (0.8, 3.7)	2.0 (1.0, 3.4)
HPV-39	1.8 (0.6, 5.2)	2.0 (0.6, 4.4)	2.0 (0.8, 3.9)	2.0 (1.0, 3.5)	1.9 (0.4, 8.4)	2.8 (0.7, 6.8)	2.6 (1.0, 5.4)	2.4 (1.1, 4.4)
HPV-40	1.7 (0.8, 3.8)	2.0 (0.9, 3.7)	2.0 (1.0, 3.5)	2.0 (1.1, 3.3)	2.6 (1.0, 6.5)	2.6 (0.9, 5.2)	2.5 (1.1, 4.7)	2.4 (1.2, 4.2)
HPV-42	1.7 (0.8, 3.4)	2.1 (1.0, 3.6)	2.0 (1.1, 3.4)	2.0 (1.2, 3.2)	1.8 (0.7, 4.7)	3.0 (1.2, 5.6)	2.8 (1.3, 5.0)	2.6 (1.4, 4.4)
HPV-43	3.4 (1.9, 6.4)	3.8 (2.0, 6.5)	3.4 (1.8, 5.5)	3.0 (1.7, 4.8)	2.0 (0.7, 5.7)	1.8 (0.6, 4.0)	1.9 (0.8, 3.7)	2.0 (1.0, 3.6)
HPV-44[b]	N/C	2.9 (0.2, 13.0)	2.2 (0.6, 5.8)	2.0 (0.8, 4.0)	N/C	3.7 (0.2, 17.7)	2.5 (0.7, 6.2)	2.3 (0.9, 4.6)
HPV-45	2.0 (0.9, 4.4)	2.0 (0.9, 3.8)	2.0 (1.0, 3.5)	2.0 (1.1, 3.3)	3.6 (1.5, 8.9)	3.7 (1.4, 7.2)	3.2 (1.4, 5.9)	2.8 (1.5, 4.9)
HPV-51	1.5 (0.6, 3.7)	2.3 (1.0, 4.4)	2.2 (1.1, 4.0)	2.1 (1.1, 3.6)	0.5 (0.1, 3.5)	1.3 (0.3, 3.2)	1.6 (0.6, 3.3)	1.8 (0.9, 3.2)
HPV-52	4.5 (2.6, 7.9)	4.3 (2.4, 7.1)	3.8 (2.2, 6.1)	3.4 (2.1, 5.3)	1.7 (0.6, 4.8)	1.6 (0.5, 3.5)	1.8 (0.8, 3.5)	1.9 (1.0, 3.3)
HPV-53	1.1 (0.1, 8.6)	1.5 (0.2, 4.5)	1.7 (0.6, 3.9)	1.8 (0.8, 3.4)	2.2 (0.3, 17.6)	2.5 (0.4, 7.6)	2.3 (0.7, 5.5)	2.3 (1.0, 4.5)
HPV-54	1.3 (0.2, 10.3)	1.6 (0.2, 4.5)	1.8 (0.6, 3.9)	1.8 (0.8, 3.4)	N/C	1.4 (0.1, 5.2)	1.9 (0.6, 4.6)	2.0 (0.9, 3.9)
HPV-55	3.8 (1.5, 9.5)	3.6 (1.3, 7.4)	3.0 (1.3, 5.7)	2.6 (1.3, 4.5)	3.0 (0.7, 13.1)	3.3 (0.8, 8.0)	2.8 (1.0, 5.9)	2.6 (1.2, 4.8)
HPV-56	3.6 (2.1, 6.2)	3.4 (1.9, 5.5)	3.1 (1.8, 4.9)	2.9 (1.8, 4.3)	1.8 (0.7, 4.5)	2.5 (1.0, 4.7)	2.4 (1.1, 4.4)	2.4 (1.3, 3.9)
HPV-57[b]	N/C	3.7 (0.2, 18.1)	2.2 (0.6, 6.2)	2.0 (0.8, 4.0)	N/C	4.2 (0.2, 20.6)	2.5 (0.7, 6.6)	2.3 (0.9, 4.7)
HPV-58	1.3 (0.6, 3.2)	1.5 (0.6, 2.9)	1.6 (0.7, 2.8)	1.7 (0.9, 2.8)	1.4 (0.4, 4.7)	1.5 (0.4, 3.5)	1.7 (0.7, 3.4)	1.9 (0.9, 3.2)
HPV-59	1.3 (0.6, 3.2)	1.5 (0.6, 3.1)	1.7 (0.8, 3.0)	1.7 (0.9, 2.9)	3.0 (1.1, 8.0)	2.6 (0.9, 5.4)	2.4 (1.1, 4.7)	2.4 (1.2, 4.2)
HPV-61[b]	N/C	3.7 (0.2, 18.1)	2.2 (0.6, 6.2)	2.0 (0.8, 4.0)	N/C	4.2 (0.2, 20.6)	2.5 (0.7, 6.6)	2.3 (0.9, 4.7)
HPV-64[b]	N/C	2.9 (0.2, 13.0)	2.2 (0.6, 5.8)	2.0 (0.8, 4.0)	N/C	3.7 (0.2, 17.7)	2.5 (0.7, 6.2)	2.3 (0.9, 4.6)

HPV-66	1.6 (0.8, 3.2)	1.7 (0.8, 3.0)	1.7 (0.9, 3.0)	1.8 (1.0, 2.9)	1.0 (0.3, 3.3)	1.2 (0.4, 2.7)	1.5 (0.6, 3.0)	1.7 (0.8, 2.9)
HPV-67	2.8 (1.5, 5.2)	3.0 (1.6, 5.0)	2.8 (1.6, 4.5)	2.6 (1.5, 4.1)	1.7 (0.6, 4.5)	1.8 (0.6, 3.6)	1.9 (0.8, 3.6)	2.0 (1.0, 3.5)
HPV-68	0.6 (0.1, 4.3)	1.1 (0.2, 3.0)	1.4 (0.5, 3.0)	1.6 (0.7, 2.9)	1.6 (0.2, 12.3)	1.8 (0.3, 5.2)	2.0 (0.7, 4.5)	2.1 (0.9, 4.0)
HPV-69	1.9 (0.5, 6.4)	1.9 (0.5, 4.6)	2.0 (0.8, 3.9)	1.9 (0.9, 3.6)	0.0 (0.0, 0.0)	0.9 (0.1, 3.1)	1.5 (0.5, 3.5)	1.8 (0.8, 3.4)
HPV-70	N/C	0.7 (0.1, 1.9)	1.1 (0.4, 2.2)	1.3 (0.6, 2.4)	1.9 (0.4, 8.1)	1.8 (0.4, 4.6)	2.0 (0.7, 4.1)	2.0 (1.0, 3.8)
HPV-71[b]	N/C	3.7 (0.2, 18.1)	2.2 (0.6, 6.2)	2.0 (0.8, 4.0)	N/C	4.2 (0.2, 20.6)	2.5 (0.7, 6.6)	2.3 (0.9, 4.7)
HPV-72	1.2 (0.2, 9.4)	1.6 (0.2, 4.8)	1.8 (0.6, 4.1)	1.8 (0.8, 3.4)	5.6 (1.2, 26.9)	4.3 (0.8, 12.4)	3.1 (1.0, 7.1)	2.7 (1.2, 5.2)
HPV-73	1.4 (0.5, 4.1)	2.1 (0.8, 4.4)	2.1 (0.9, 4.0)	2.0 (1.0, 3.6)	1.9 (0.4, 8.2)	2.6 (0.7, 6.2)	2.4 (0.9, 5.0)	2.3 (1.1, 4.2)
HPV-81	3.9 (1.9, 8.4)	3.7 (1.6, 7.0)	3.1 (1.5, 5.5)	2.8 (1.5, 4.7)	0.6 (0.1, 4.9)	1.1 (0.2, 3.1)	1.6 (0.5, 3.4)	1.8 (0.8, 3.3)
HPV-82	1.3 (0.2, 10.3)	1.6 (0.3, 5.0)	1.8 (0.6, 4.2)	1.8 (0.8, 3.5)	5.6 (1.2, 26.7)	4.5 (0.8, 12.7)	3.2 (1.0, 7.4)	2.7 (1.2, 5.2)
HPV-83	1.2 (0.3, 5.4)	1.9 (0.5, 4.5)	1.9 (0.8, 3.8)	1.9 (0.9, 3.5)	3.9 (1.1, 13.7)	3.6 (0.9, 8.7)	2.9 (1.0, 6.2)	2.6 (1.2, 4.8)
HPV-84	N/C	1.3 (0.1, 4.4)	1.6 (0.5, 3.9)	1.8 (0.8, 3.5)	N/C	1.8 (0.2, 6.8)	2.1 (0.6, 5.0)	2.1 (0.9, 4.3)
HPV-85	3.2 (0.4, 28.0)	3.3 (0.4, 11.1)	2.4 (0.7, 5.9)	2.1 (0.9, 4.3)	N/C	2.1 (0.2, 8.5)	2.2 (0.6, 5.4)	2.2 (0.9, 4.5)
HPV-86	N/C	1.1 (0.1, 3.8)	1.5 (0.5, 3.7)	1.7 (0.7, 3.2)	N/C	3.3 (0.4, 10.8)	2.6 (0.8, 6.1)	2.4 (1.0, 4.7)
HPV-89	0.9 (0.2, 3.7)	1.1 (0.2, 2.8)	1.4 (0.5, 2.8)	1.5 (0.7, 2.8)	0.9 (0.1, 7.0)	1.3 (0.2, 3.8)	1.7 (0.6, 3.7)	1.9 (0.9, 3.5)
HPV-JC9710	1.3 (0.5, 3.1)	1.2 (0.5, 2.4)	1.4 (0.7, 2.4)	1.5 (0.8, 2.5)	2.2 (0.8, 5.9)	2.1 (0.7, 4.4)	2.1 (0.9, 4.1)	2.1 (1.1, 3.7)
HPV-X	1.5 (0.8, 3.1)	1.7 (0.8, 2.9)	1.7 (0.9, 2.8)	1.7 (1.0, 2.7)	0.3 (0.0, 2.3)	0.6 (0.1, 1.5)	1.0 (0.4, 2.0)	1.3 (0.6, 2.3)

Abbreviations: N/C (sparse or no data, could not calculate estimate)

[a]Odds ratios for pair-wise associations are adjusted for age, travel to Nairobi (in 6 months), bathing frequency, number of sexual partner (in 6 months), consistent condom use (in 6 months), and current Gonorrhea and *C. trachomatis* infection

[b]HPV-57, HPV-61, HPV-71 no infections; HPV-34, HPV-64: no single infections; HPV-44 no multiple infections

Appendix Table 5

Estimated odds ratios between the vaccine-preventable HPV types 16 and 18 and all other HPV types including HPV-6 and 11

	HPV-16 aOR (95% CI)[a]	HPV-18 aOR (95% CI)[a]
HPV-6	1.7 (1.1, 2.6)	1.9 (1.1, 3.1)
HPV-11	2.0 (1.1, 3.2)	1.8 (0.9, 3.1)
HPV-26	1.7 (0.8, 3.0)	4.3 (2.1, 7.6)
HPV-30	1.7 (0.9, 2.9)	3.0 (1.5, 5.3)
HPV-31	2.9 (1.9, 4.3)	2.8 (1.6, 4.4)
HPV-32	1.7 (0.9, 3.0)	2.2 (1.1, 3.9)
HPV-33	1.9 (1.0, 3.0)	2.5 (1.3, 4.4)
HPV-34	2.0 (0.8, 4.0)	3.0 (1.2, 6.3)
HPV-35	2.6 (1.7, 3.8)	1.9 (1.1, 3.1)
HPV-39	2.1 (1.2, 3.5)	2.2 (1.1, 3.9)
HPV-40	3.7 (2.4, 5.4)	2.2 (1.3, 3.5)
HPV-42	2.1 (1.4, 3.1)	2.2 (1.3, 3.5)
HPV-43	1.8 (1.1, 2.7)	2.3 (1.3, 3.6)
HPV-44	2.0 (0.9, 4.2)	2.6 (1.1, 5.3)
HPV-45	1.8 (1.1, 2.7)	2.9 (1.6, 4.6)
HPV-51	2.3 (1.4, 3.4)	1.8 (1.0, 3.0)
HPV-52	1.7 (1.1, 2.6)	2.3 (1.3, 3.7)
HPV-53	2.6 (1.3, 4.5)	2.2 (1.0, 4.1)
HPV-54	1.4 (0.7, 2.6)	2.5 (1.1, 4.7)
HPV-55	1.5 (0.8, 2.6)	2.0 (1.0, 3.6)
HPV-56	2.2 (1.5, 3.1)	1.8 (1.1, 2.9)
HPV-57	2.1 (0.8, 4.3)	2.7 (1.1, 5.4)
HPV-58	2.6 (1.7, 3.9)	2.2 (1.3, 3.6)
HPV-59	2.4 (1.5, 3.6)	2.9 (1.6, 4.6)
HPV-61	2.1 (0.8, 4.3)	2.7 (1.1, 5.4)
HPV-64	2.0 (0.9, 4.2)	2.6 (1.1, 5.3)
HPV-66	2.1 (1.3, 3.0)	2.3 (1.3, 3.6)
HPV-67	2.4 (1.6, 3.5)	1.8 (1.0, 2.9)
HPV-68	1.3 (0.6, 2.3)	2.1 (1.0, 3.7)
HPV-69	2.5 (1.4, 4.2)	2.8 (1.4, 4.9)
HPV-70	1.6 (0.9, 2.6)	1.7 (0.8, 3.0)
HPV-71	2.1 (0.8, 4.3)	2.7 (1.1, 5.4)
HPV-72	2.7 (1.3, 4.9)	2.6 (1.2, 4.8)
HPV-73	2.2 (1.2, 3.6)	3.0 (1.6, 5.0)
HPV-81	1.7 (1.0, 2.8)	2.4 (1.2, 4.0)

HPV-82	1.5 (0.7, 2.7)	2.3 (1.0, 4.4)
HPV-83	1.8 (0.9, 3.0)	2.8 (1.4, 4.8)
HPV-84	1.8 (0.8, 3.3)	2.2 (0.9, 4.2)
HPV-85	2.0 (0.8, 3.9)	2.7 (1.2, 5.5)
HPV-86	1.8 (0.9, 3.3)	3.1 (1.4, 5.9)
HPV-89	1.8 (1.0, 2.9)	2.4 (1.2, 4.1)
HPV-JC9710	1.8 (1.1, 2.7)	2.8 (1.6, 4.3)
HPV-X	1.2 (0.8, 1.8)	1.7 (1.0, 2.7)

Abbreviations: aOR (adjusted odds ratio); CI (confidence interval)
[a]Odds ratios for pair-wise associations are adjusted for age, travel to Nairobi (in 6 months), bathing frequency, number of sexual partner (in 6 months), consistent condom use (in 6 months), and current Gonorrhea and *C. trachomatis* infection

Appendix Table 6

Comparison of any-HPV acquisition results for HPV-positive restricted population compared to analysis of the full cohort

	Restricted population: HPV positive at least once during study period		**Unrestricted:** Original, full cohort	
	aHR (95% CI) [a,b]	aMSR (95% CI) [a,b]	aHR (95% CI) [a,b]	aMSR (95% CI) [a,b]
HPV-16	1.0 (0.8, 1.3)	1.0 (0.8, 1.3)	1.2 (0.9, 1.6)	1.2 (0.9, 1.7)
HPV-18	1.7 (1.2, 2.5)	1.7 (1.2, 2.6)	2.1 (1.4, 3.3)	2.2 (1.4, 3.5)
HPV-31	1.3 (0.9, 1.8)	1.3 (0.9, 1.9)	1.5 (1.0, 2.3)	1.6 (1.0, 2.4)
HPV-45	1.6 (1.2, 2.2)	1.7 (1.2, 2.3)	2.0 (1.4, 2.8)	2.1 (1.4, 3.1)
HPV-6	1.2 (0.8, 1.7)	1.2 (0.8, 1.8)	1.4 (0.9, 2.1)	1.4 (0.9, 2.2)
HPV-11	1.4 (0.9, 2.4)	1.5 (0.9, 2.5)	1.8 (1.0, 3.1)	1.8 (1.0, 3.3)
HPV-16, 18[c]	1.2 (1.0, 1.5)	1.2 (1.0, 1.5)	1.5 (1.2, 1.9)	1.5 (1.2, 2.0)
HPV-6, 11[d]	1.3 (0.9, 1.7)	1.3 (0.9, 1.8)	1.5 (1.0, 2.1)	1.5 (1.0, 2.2)
HPV-16, 18, 6, 11[e]	1.2 (1.0, 1.5)	1.2 (1.0, 1.5)	1.5 (1.2, 1.9)	1.5 (1.2, 1.9)

Abbreviations: aHR (adjusted hazard ratio); CI (confidence interval); aMSR (adjusted median survival ratio)

[a]Parametric frailty models adjusted for age, bathing frequency, number of sexual partners (in 6 months), consistent condom use (in 6 months), *C. trachomatis* infection

[b]All other HPV types except exposure HPV type

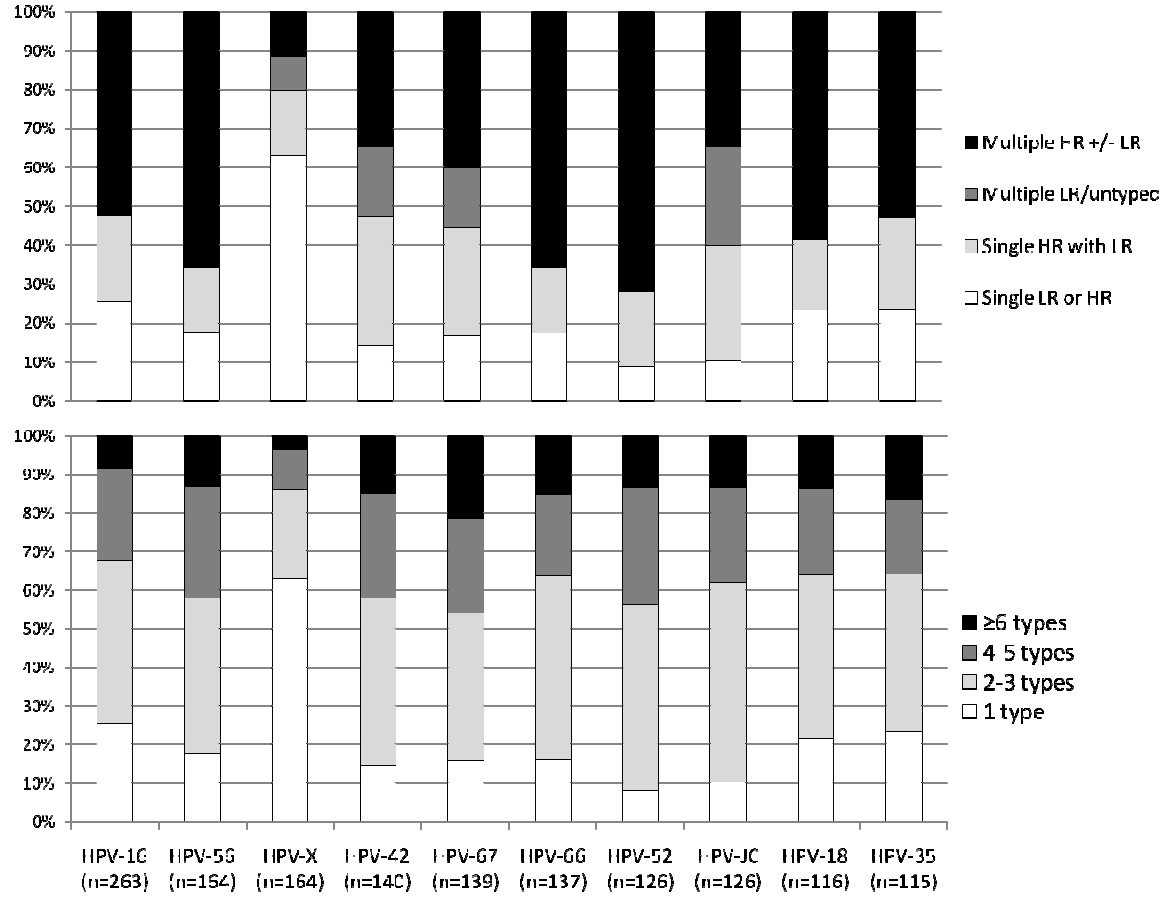
[c]Index group HPV-16 and/or 18 positive vs. referent group HPV-16 and 18 negative

[d]Index group HPV-6 and/or 11 positive vs. referent group HPV-6 and 11 negative

[e]Index group HPV-16, 18, 6, and/or 11 positive vs. referent group HPV-16, 18, 6, and 11 negative

Appendix Figure 1

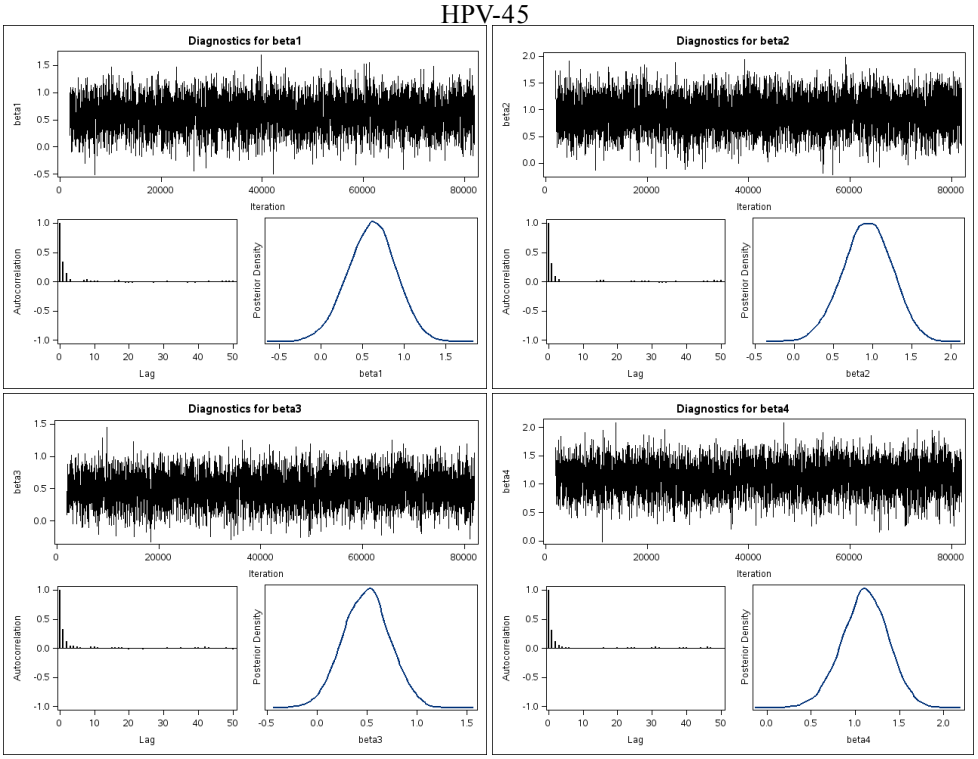
Distribution of single and multiple type infections in the 10 most prevalent HPV types



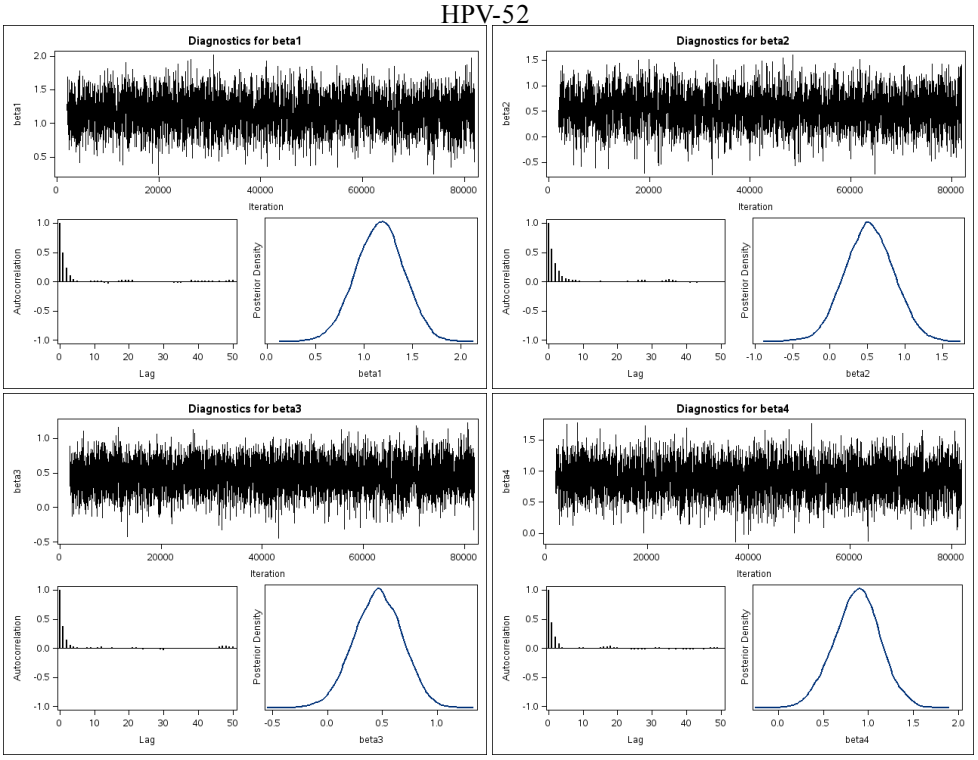
Appendix Figure 2

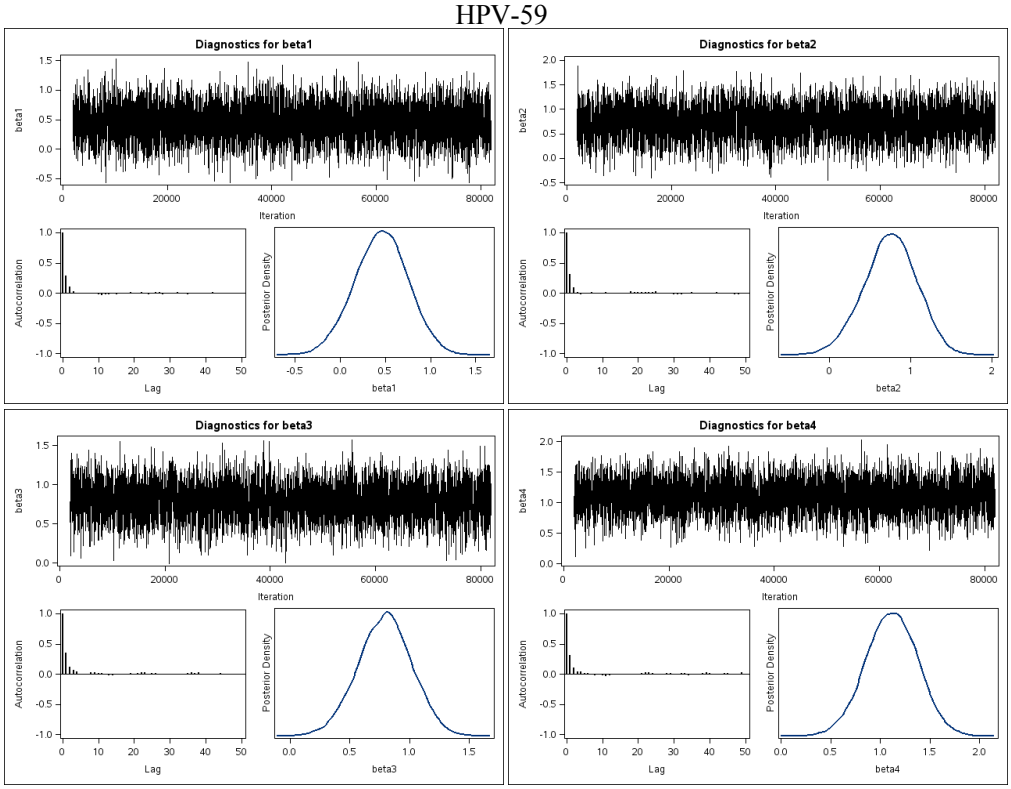
Select diagnostic plots from PROC MCMC

HPV-45



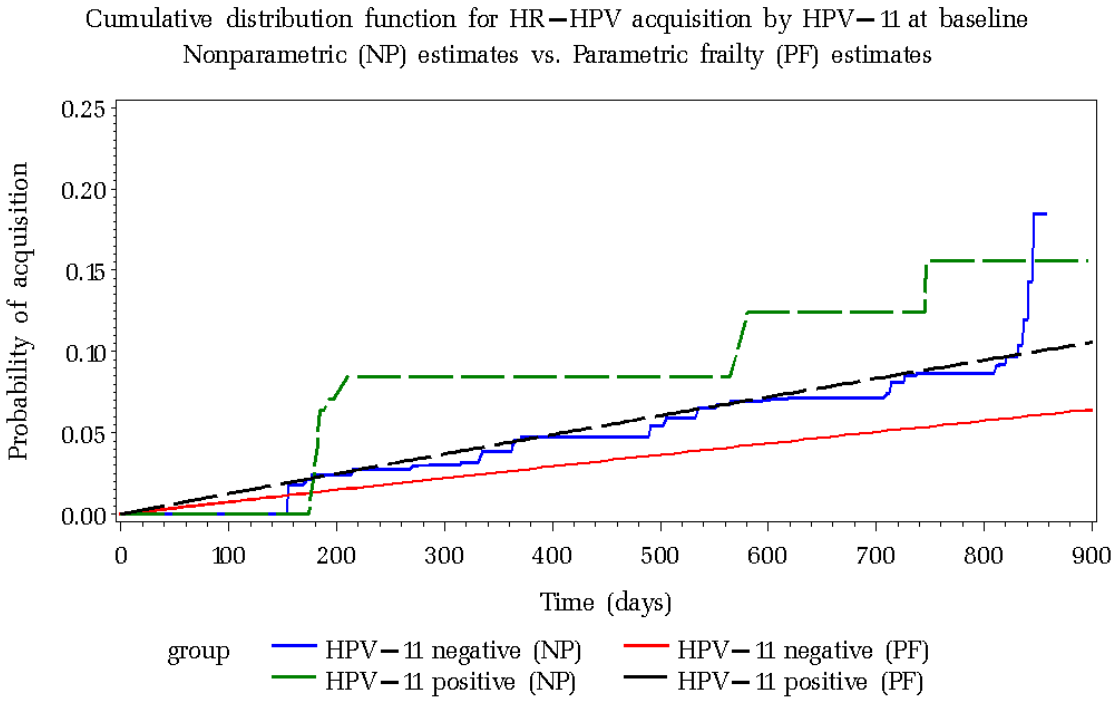
HPV-52

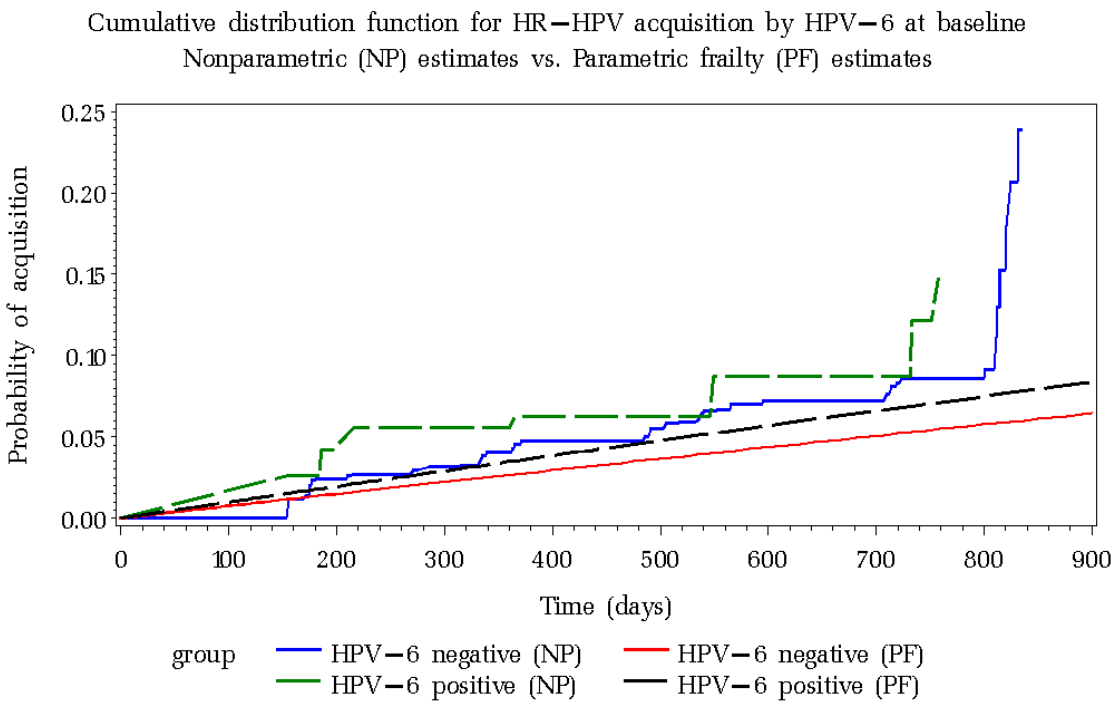


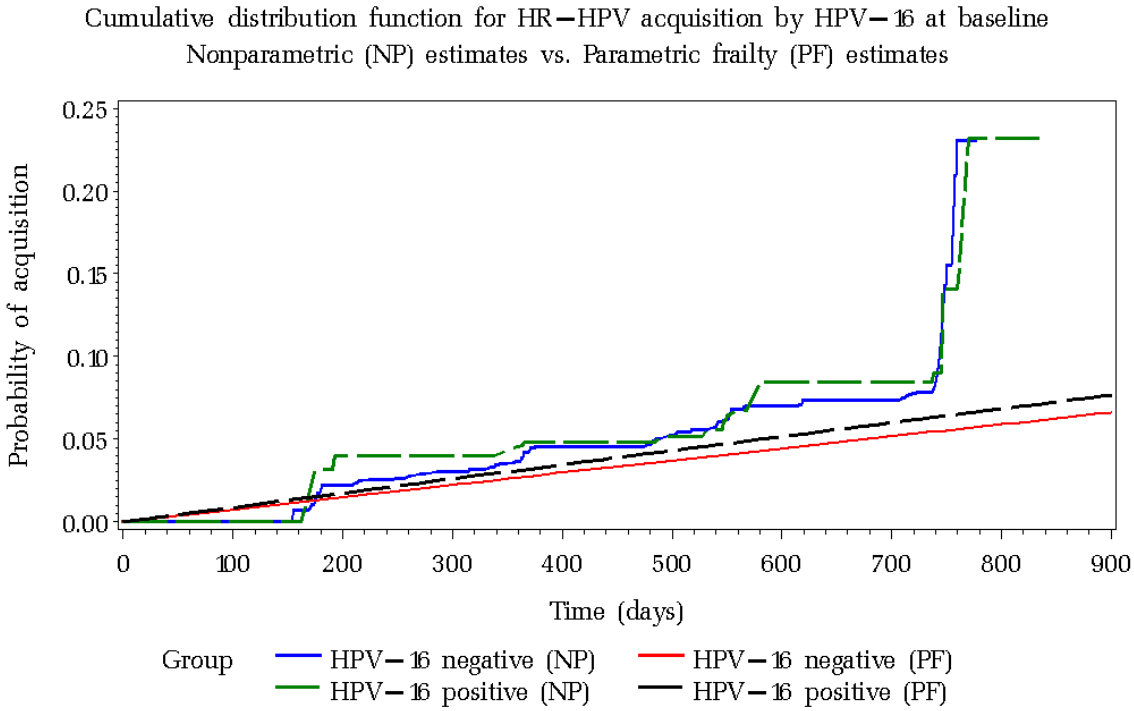

Diagnostics for beta1
beta1
Iteration
Autocorrelation
Lag
Posterior Density
beta1
Diagnostics for beta2
beta2
Iteration
Autocorrelation
Lag
Posterior Density
beta2
Diagnostics for beta3
beta3
Iteration
Autocorrelation
Lag
Posterior Density
beta3
Diagnostics for beta4
beta4
Iteration
Autocorrelation
Lag
Posterior Density
beta4

Appendix Figure 3

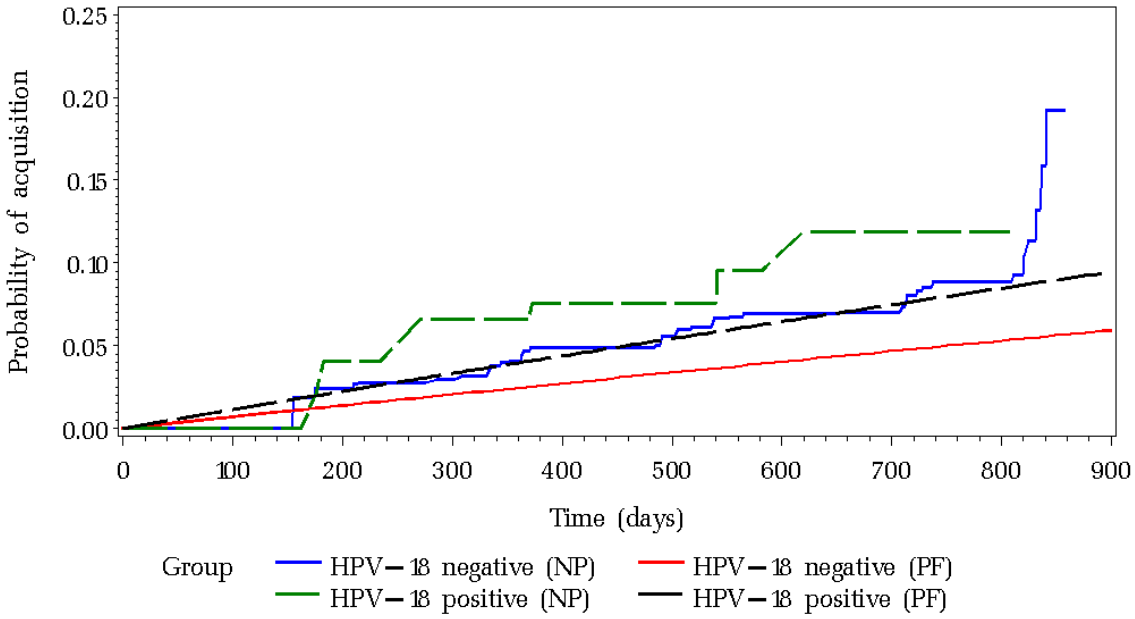
Survival function curves for HR-HPV acquisition estimated by nonparametric (NP) and parametric frailty (PF) survival models



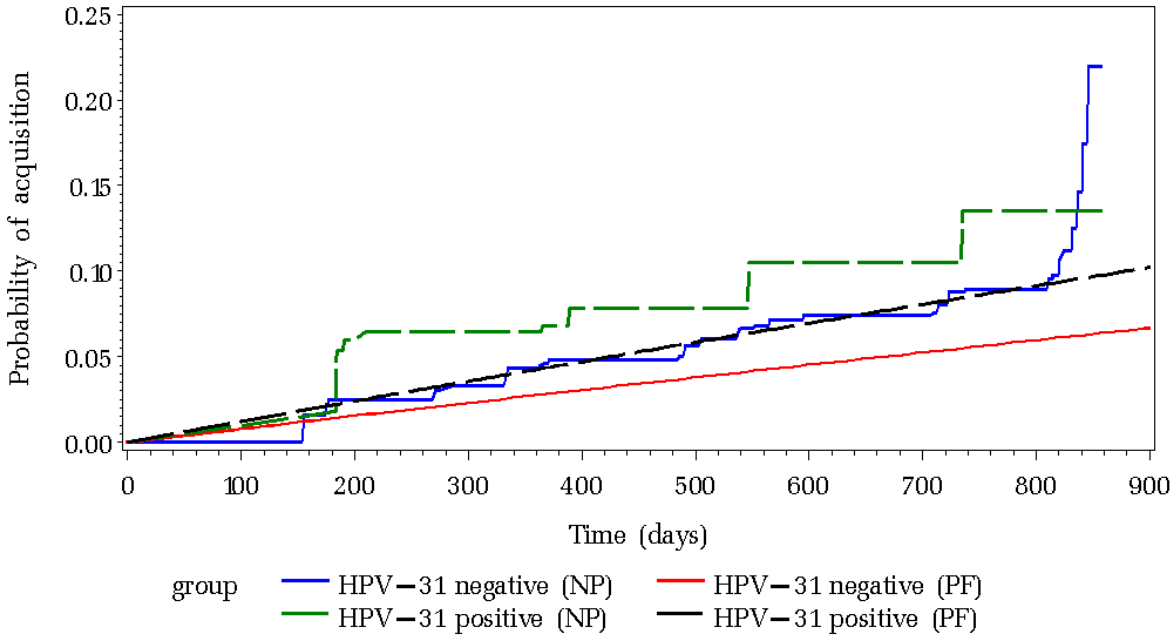

Cumulative distribution function for HR−HPV acquisition by HPV−6 at baseline
Nonparametric (NP) estimates vs. Parametric frailty (PF) estimates
Probability of acquisition
0.25
0.20
0.15
0.10
0.05
0.00
0
100
200
300
400
500
600
700
800
900
Time (days)
group
HPV−6 negative (NP)
HPV−6 positive (NP)
HPV−6 negative (PF)
HPV−6 positive (PF)

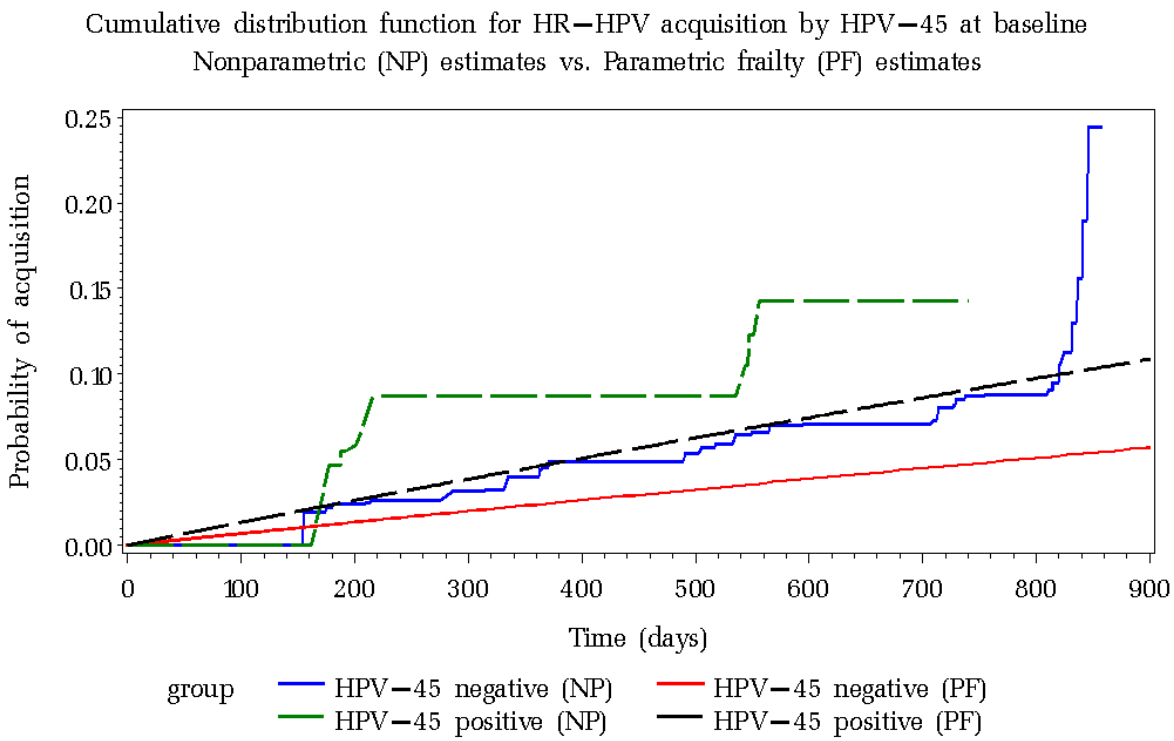

Cumulative distribution function for HR−HPV acquisition by HPV−16 at baseline
Nonparametric (NP) estimates vs. Parametric frailty (PF) estimates
Probability of acquisition
0.25
0.20
0.15
0.10
0.05
0.00
0
100
200
300
400
500
600
700
800
900
Time (days)
Group
HPV−16 negative (NP)
HPV−16 positive (NP)
HPV−16 negative (PF)
HPV−16 positive (PF)

Cumulative distribution function for HR−HPV acquisition by HPV−18 at baseline
Nonparametric (NP) estimates vs. Parametric frailty (PF) estimates



Cumulative distribution function for HR−HPV acquisition by HPV−31 at baseline
Nonparametric (NP) estimates vs. Parametric frailty (PF) estimates




Cumulative distribution function for HR−HPV acquisition by HPV−45 at baseline
Nonparametric (NP) estimates vs. Parametric frailty (PF) estimates
Probability of acquisition
0.25
0.20
0.15
0.10
0.05
0.00
0
100
200
300
400
500
600
700
800
900
Time (days)
group
HPV−45 negative (NP)
HPV−45 positive (NP)
HPV−45 negative (PF)
HPV−45 positive (PF)

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