3-15-2016

Factors Associated with Sexually Transmitted Infections (STIs) and Multiple STI Co-infections: Results from the EVRI HIV Prevention Preparedness Trial

Ubin Pokharel

Factors Associated with Sexually Transmitted Infections (STIs) and Multiple STI Co-infections: Results from the EVRI HIV Prevention Preparedness Trial

by

Ubin Pokharel

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Public Health
Department of Epidemiology and Biostatistics
College of Public Health
University of South Florida

Co-Major Professor: Yougui Wu, Ph.D.
Co-Major Professor: Anna Giuliano, Ph.D.
James Mortimer, Ph.D.
Lynette Menezes, Ph.D.
Staci Sudenga, Ph.D.

Date of Approval:
March 11, 2016

Keywords: HPV, STI Co-infections, Chlamydia, HSV-2, HPV Vaccines

Table of Contents

List of Tables

List of Figures

Abstract

Objective: The objective of this study was to describe the prevalence of sexually transmitted co-infections and assess factors associated with a single infection and co-infections.

Methods: A total of 388 women were included in this study. At enrollment of the EVRI trial women were tested for five STIs: Human papilloma virus (HPV), *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, Herpes simplex virus-2 (HSV-2) and *Treponema pallidum*. Prevalence of STI infections and proportion of women with different combinations of co-infections were calculated. Factors associated with single infection and STI co-infection were assessed using a polytomous logistic regression model and odds ratio (OR) and 95% confidence intervals (95% CI) were reported as the measure of association.

Results: Prevalence of a single STI and concurrent STI co-infections were high. The most common STI co-infection pattern was HPV-HSV (32%), followed by HPV-chlamydia (17%). The odds of a single STI compared to no STIs were significantly higher (OR 3.02, 95% CI: 1.05-8.64) and the odds of concurrent STIs compared to no infection were significantly higher (OR 3.86, 95% CI: 1.42-10.48) for women with three or more lifetime partners compared to one life time partner.

Conclusions: STIs, single and multiple concurrent infections, are common among this cohort of South African women. These results strengthen the recommendation that STI screening and treatment needs to be a component of multiple intervention strategies among high-risk women residing in communities with high STI prevalence.

Introduction

Sexually transmitted infections (STIs) are a major public health concern in many developing countries. Women are more likely to bear the burden of these infections but less likely to seek medical treatment.[1] STIs are responsible for severe adverse reproductive outcomes and published reports from the World Health Organization (WHO) suggest that Africa remains the continent worst affected.[2] Human-papilloma virus (HPV) causes cervical cancer, and other STIs like *Chlamydia trachomatis* (chlamydia) and *Treponema pallidum* (syphilis) are major causes of both maternal and childhood morbidity during pregnancy.[3] High prevalence of STIs in many developing countries has been attributed to a multifactorial etiology where there is a complex interplay of demographic, socio-economic, and sexual behavioral factors.[4]

Current Epidemiology of HPV

HPV infection, a double stranded DNA virus, is the most common STI and a major public health issue globally. Currently more than 100 different HPV genotypes have been identified, and of these 13 genotypes (HPV 16,18,31,33,35,39,45,51,52,56,58,59 and 68) have been designated high-risk (HR-HPV) or carcinogenic due their potential to cause high-grade intraepithelial lesions (HSIL's), the precursor to cervical cancer, and cancer. Most women naturally clear HPV infections (low-risk HPV 12-month clearance 12.2%, 95% confidence interval (CI): 9.6%-15.4%, but some HR-HPV genotypes, especially HPV 16, tend to persist (12-month clearance 9.5%, 95% CI: 7.5%-11.9% and may lead to neoplasia.[5] Previous studies have shown that co-infections with other STIs lead to persistence of HR-HPV infections.[6] A meta-analysis of studies worldwide that included nearly 160,000 females with normal cervical cytology had ~10% HPV prevalence and the highest regional cervical HPV prevalence was 22% among women in Africa.[7] HPV infection

accounts for 100% of invasive cervical cancers, and nearly 70% of these cancers are caused by HPV genotypes 16 and 18.[8] Apart from cervical cancer, approximately 90% of anal, 43% of vulvar, 70% of vaginal and nearly 50% of penile cancers are attributed to a persistent HPV infection.[9] Among women in rural sub Saharan Africa, one study reported a cervical HPV prevalence of 40%.[8]

It has been suggested that there is a rapid cervical HPV and STI co-infection acquisition after sexual debut. HPV vaccines are prophylactic and are therefore recommended prior to sexual debut, typically ages 9-12.[10] There are currently three HPV vaccines (Gardasil, Gardasil 9, and Cervarix) that have shown efficacy in preventing cervical disease. Data from the Centers for Disease Control and Prevention (CDC) indicate that there is nearly 56% reduction in HPV infections among teenage girls in the United States since the inception and commercial use of the HPV vaccines.[11] The 9-valent vaccine is estimated to prevent anogenital warts and 90% of cervical disease by providing protection from HPV 6,11,16,18,31,33,45,52 and 58.[9]

Current Epidemiology of other STIs

The WHO estimates that globally, there are about 340 million new cases of the four main curable STIs (Chlamydia, gonorrhea, syphilis and trichomoniasis) each year.[12]

Chlamydia trachomatis, an intracellular bacterium, is the most common bacterial STI in the United States and globally.[13] It commonly affects the urethra in males and the urethra and the cervix in females. Presence of these bacteria in the cervix is an important risk factor for persistent HPV infection and ascending chlamydial infections are common in females that can ultimately contribute to ectopic pregnancy and infertility.[6] Most cases of genital chlamydia are asymptomatic, only identified through screening procedures, providing a constant source of efficient disease transmission. In a study carried out by CDC, the prevalence of chlamydia infection in persons aged 14-39 years was 1.7% (95% CI 1.4%-2.0%). However, the prevalence among sexually active females aged 14-24 years was 4.7% and 13.5% among non-Hispanic black

females.[14] The WHO reports that there were nearly 100 million prevalent cases of chlamydia and nearly 105 million incident cases worldwide in 2008 which represents a 5% increase from 2005.[2] Prevalence of chlamydial infection varies from 2.1% (95% CI 1.6-4.1) among women aged 20-64 years in China [15] to 8% (95% CI 5%-11%) among women in rural sub-Saharan Africa [8], and 19.5% (95% CI 14.9%-24.1%) among women in South Africa.[16]

Neisseria gonorrhoeae, a gram-negative diplococci, is the second most prevalent bacterial STI and is the second most commonly reported infectious disease in the USA.[13] It can cause either a localized pelvic infection or a generalized systemic infection called disseminated gonococcal infection (DGI). It is commonly found in the urethra in males and the cervix in females. The WHO estimated the global incidence of gonorrhea in 2008 to be 106 million cases representing a 21% increase from 2005.[2] However, an accurate estimation of gonorrhea incidence is difficult because of the lack of diagnostic and reporting capabilities around the world. The highest gonorrhea prevalence was seen in Africa, ranging from 1.5% in west and central Africa to 4.9% in east and southern Africa. In one study among women in rural sub-Saharan Africa, gonorrhea prevalence was 14% (95% CI 10%-19%) [8], and 16.5% (95% CI 12.9%-20.2%) in South Africa.[16] A population-based study in China reported 0.08% (95% CI 0.02-0.4) prevalence among women aged 20-64 years.[2] The incidence of gonorrhea in the United States was 106 cases per 100,000 persons in 2013, with the incidence 12 times higher in blacks compared to whites (427 vs 35 per 100,000 persons). For women, the incidence was highest for those aged 20-24 years (542 cases per 100,000 persons).[13] Gonorrhea incidence has been higher in males, as the disease in more asymptomatic in females and possibly due to higher incidence of gonorrheal infection in men having sex with men.

Treponema pallidum, a gram-negative spirochete, causes syphilis characterized by a symptomatic early stage and an asymptomatic latent stage. Most syphilis research is focused on the early stage, since transmission is unlikely in the latent stages. The estimated syphilis

incidence in the United States was 5.3 cases per 100,000 persons in 2013 representing an increase from 2.9 cases per 100,000 in 2005.[13] There is a major racial difference in syphilis incidence in the US, with the incidence being 5.2 times higher in blacks compared to whites (27.9 vs 5.4 per 100,000 persons). The incidence among women was 0.9 per 100,000 and black women were 13.3 times more likely to have incident syphilis infection compared to white women (4.0 vs 0.3 per 100,000 person).[13] The WHO reports that there were nearly 12 million incident cases of syphilis worldwide in 2008, with highest incidence seen in South East Asia and Africa.[2] Prevalence of syphilis varies from 12% (95% CI 8%-17%) [8] among women in rural sub-Saharan Africa to 7.5% (95% CI 5.2%-9.8%) [16] in South Africa. There is a high rate of HIV co-infection in people who are infected with syphilis and most cases of primary and secondary syphilis occur among MSM.[13]

Herpes Simplex Virus-2 (HSV-2), a double strand DNA virus, is ubiquitous and a highly contagious virus that is transmitted by contact with an infected area. HSV-2 seropositivity has emerged as a major public health issue in sub-Saharan Africa as HSV-2 infection increases the risk of HIV transmission and acquisition.[17, 18] Similarly, HSV-2 seropositivity has been proposed to be a marker of sexual risk behaviors in young adults.[17] There are around 50 million people who are seropositive for HSV-2 in the US.[13] The age-adjusted prevalence of HSV-2 in the US is 17% (95% CI 15.8%-18.3%), however genital HSV is often undiagnosed as it is mostly asymptomatic.[19] The seroprevalence of HSV-2 increases with age, number of sexual partners, and is higher in women compared to men.[19] As with other STIs there is a racial difference in the prevalence of HSV-2 with it being three times higher in blacks compared to whites (39% vs 12%).[13] The WHO reports that a higher prevalence of HSV-2 is seen in Africa and South East Asia and the lowest prevalence is seen in Europe (18% of women and 13% of men).[2] A study performed among women in rural sub- Saharan Africa showed an extremely high HSV-2 prevalence of 83% (95% CI 78%-87%) [8], with the seroprevalence increasing with age.

Factors associated with STIs

Age: Age has been associated with the risk of STI, however there are study specific variations on STIs influenced by age. A study among women in rural sub Saharan Africa reported gonorrhea infection being more prevalent in the younger age group, 14-20 years (P<0.01),[16] whereas there was no age-related association with chlamydia. Syphilis showed increased prevalence with age but the association was not statistically significant (P=0.67). The frequency of HSV-2 antibodies increased significantly with age (P<0.01) and the lowest prevalence was seen in the youngest age group. Prevalence of LR-HPV and HR- HPV decreases with age in women, with the prevalence being highest in the youngest age group, 14-20 years (P<0.001).[8] Among migrant workers in South Africa age was associated with the risk of STI, but the effect of age diminished when migration status was considered [4].Initial analysis of the current EVRI trial showed that prevalence of HPV was highest among youngest women, aged 16-17 and decreased with age. Prevalence of gonorrhea and chlamydia were highest in younger women and significantly decreased with age. The prevalence of syphilis was highest at age 16 (20%) and was significantly lower among women aged 17-24 (3-10%).[10]

Sociodemographic and Behavioral Risk Factors: Education, marital status, age at first sexual intercourse, life-time number of male sexual partners, and number of new sexual partners have all been established as important risk factors that determine the prevalence of STIs. Women who reported more than one lifetime partner had higher risk of cervical gonorrhea infection (odds ratio (OR) 2.8, 95%CI: 1.2-6.2). Women who were divorced or widowed had higher risk of syphilis (OR 3.6, 95% CI: 0.6-21.9) and South African women who were single (OR 5.6, 95% CI: 1.8-17.3) and who were living with a partner (OR 2.3, 95% CI: 1.3-4.1) had significant higher risk of chlamydia infection.[8] One prospective study showed that having more than two sexual partners in the past increased the risk of acquiring chlamydia (OR 19.2, 95%CI: 1.2-296.7) and gonorrhea (OR 3.4 95%CI: 0.3-33.2) infection.[16] In South Africa, the risk of acquiring chlamydia (OR 1.7, 95% CI:

1.3-4.1) and gonorrhea (OR 2.0, 95% CI: 1.2-3.4) was higher for women who had completed secondary or higher level of school education [16] and for women who had received money or some form of compensation for the sexual activity. Migrant men (OR 1.54, 95% CI: 1.0-2.3), who leave their home primarily for better paying jobs, and their partners (OR 1.2, 95% CI: 0.7-1.88) also reported a higher risk of contracting a STI.[4]

Co-infection Status: Co-infection is believed to act bi-directionally whereby being infected with one STI increases the risk of acquiring another STI. Women who were HIV-positive were more likely to have HPV DNA detected at the cervix (47% vs 37%, OR 1.6, 95% CI: 0.5-3.5). It was also shown that women who tested positive for syphilis were more likely to test positive for HPV DNA (OR 4.1, 95% CI: 1.9-9.1).[8] Presence of chlamydia or gonorrhea infection was also associated with higher likelihood of HSV-2 antibody positivity (OR 2.4, 95% CI: 1.1-5.4) and the presence of either of gonorrhea or syphilis increased the likelihood of being infected with chlamydia infection (OR 2.3, 95% CI: 1.0-5.1).[8] A study in South Africa reported that being positive for active syphilis increased the likelihood of acquiring gonorrhea (OR 4.0, 95%CI: 1.2-13.3), being positive for gonorrhea increased the likelihood of acquiring chlamydia (OR 4.7, 95%CI: 2.4-9.5) and being positive for chlamydia increased the likelihood of acquiring gonorrhea (OR 5.0, 95%CI: 2.5-9.9). Having an incident HIV infection significantly increased the likelihood of getting a chlamydia (OR 7.3, 95%CI: 2.9-18.6) and a gonorrhea infection (OR 5.5, 95%CI: 1.9-16.3).[16] Synergy between STI risk factors, particularly sexual behavior and biological mechanisms like mucosal disruption, alteration of organism's natural history could provide explanation as to why women with one STI are more likely to have infection with another STI.[20]

Although most of the studies suggest HIV infection increases risk of acquiring other STIs, recent studies among men and women have found an increased risk of HIV among HPV-positive individuals [15] and it is possible that a portion of HIV infections could be prevented through HPV vaccination. The **E**fficacy of HPV **V**accine to **R**educe HIV **I**nfection (EVRI) is a placebo controlled

Phase II Trial that was carried out to assess the feasibility of a HPV vaccine trial in a female population aged (16-24) living in the Western Cape, South Africa who are at high risk for HPV and HIV infections. The secondary aim of this trial was to estimate the prevalence of HIV, HPV and other sexually transmitted infections (STI) by age. The EVRI trial showed high rates of accrual of eligible females (93%) and high completion rates of the three-dose HPV vaccine series (91%) among 402 sexually active HIV-negative females.

Thesis Specific Aims

Given that women in South Africa share a high burden of HIV and sexually transmitted infections, the first objective was to describe the prevalence of STI co-infections (HPV, chlamydia, gonorrhea, syphilis and HSV-2) among young women from the Western Cape at enrollment in the EVRI trial as well as the proportion of women with the different combinations of co-infections. The second objective was to assess factors associated with a single infection and multiple sexually transmitted co-infections.

Methods

Population

The EVRI trial was conducted in the Western Cape, South Africa among women ages 16-24 who were enrolled in the study from November 2012 to July 2013.[10] Participants were recruited using brochures, flyers and word of mouth, inviting them to participate in a vaccine research study against cervical cancer. Two clinic sites, Kraaifontein day hospital and Bloekombos primary health care clinic, were used as the recruitment centers. These study sites were chosen because of previous clinical data that indicated high HIV prevalence as well as the availability of infrastructure to carry out this trial. Participants were informed about the link between HIV and HPV. They were also informed that the study results would be used to determine the necessity of a larger future trial to evaluate the potential role of HPV vaccine in preventing HIV infection. Women who participated in the study were compensated for their time and transportation with an aim of improving compliance in the study.

Participants who showed interest in the study were screened based on the eligibility criteria (Appendix A). Those who did not meet eligibility requirements or those who were unwilling and later dropped out were excluded from the study. Briefly, HIV-negative women aged 16-24 years, not pregnant, without a history of abnormal cervical Pap smear, and not enrolled in any other HIV trial were eligible for enrollment into this study. A total of 924 women were approached to participate in this trial. 404 of these women were not enrolled in the study due to ineligibility and an additional 41 either became unwilling to participate or dropped out of the study (Figure 1). 479 women were enrolled into the study who met eligibility after administration of the questionnaire (Appendix A). HIV and pregnancy testing at enrollment was conducted. Twelve women were

pregnant, 57 tested positive for HIV, 1 was both pregnant and HIV positive. These 70 women were not randomized due to trial ineligibility. Seven women dropped out of the trial, which left 402 women who were randomized to receive the vaccine or placebo. Women who were pregnant were referred for related medical care and were excluded from the study.

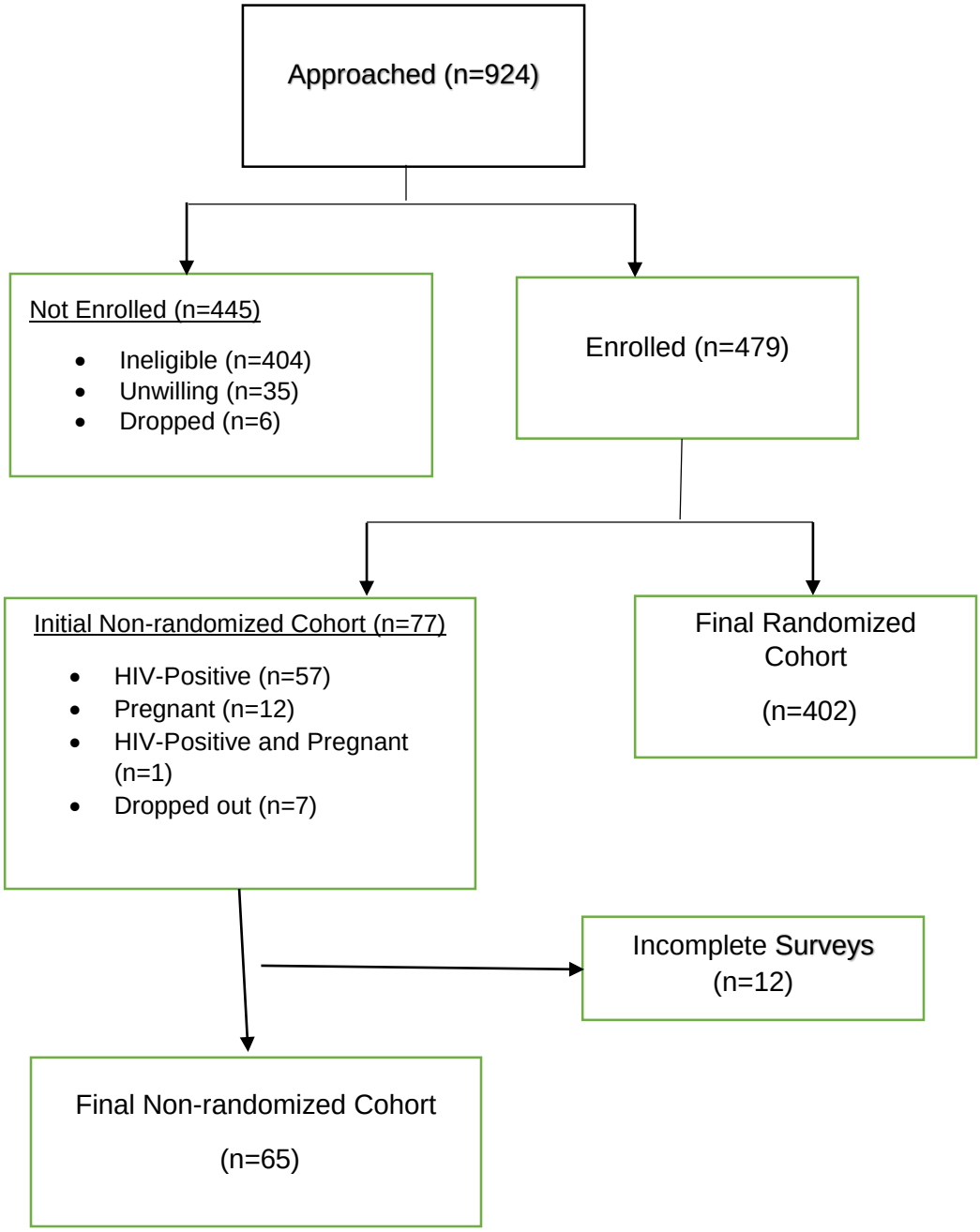


Figure 1. The EVRI Study: Study Protocol Flowchart

This trial was performed in accordance with the ethics committee review and was approved by the Institutional review boards of USF (University of South Florida) and Stellenbosch University, South Africa. Parental consent was obtained for children (aged 16-17 years) participating in this trial and getting tested for HIV. Parents were informed about their child's HIV test results.

Study Protocol

During the enrollment phase of the EVRI trial, participants were asked about their health, socio-demographic and sexual history using a tablet-based questionnaire that was available in three regional languages (English, Xhosa & Afrikaans). The tablet-based questionnaire was a source of initial confusion in the trial as 116 participants reported not having had vaginal intercourse during the eligibility screening. Later, in-depth qualitative interviews with the participants revealed the confusion was with the term "vaginal". Different types of intercourse was explained to the participants to prevent misinterpretation in reported sexual history. A similar tablet-based questionnaire was used during the month 7 visit to record health, socio-demographic and sexual history.

As described, the EVRI trial was a Phase II randomized controlled trial involving the 4-valent HPV vaccine (Gardasil). Women were randomized 1:1 to receive either the Gardasil vaccine or placebo (saline). The EVRI trial was double blinded study where the participant's vaccination status was unknown to the staff and study investigators with the exception of the pharmacist who allocated the vaccines. The vaccine and placebo were administered at enrollment (baseline), month 2 and month 6. Participants were followed for 1 month (Month 7) after the third vaccine dose, when unblinding of the vaccination status occurred and the study participants who were in the placebo arm of the trial were offered the chance of receiving Gardasil at no charge.

Gonorrhea and chlamydia were tested using urine samples at enrollment and Month 7. Herpes Simplex Virus (HSV)-2 antibodies and syphilis testing was measured in the participant's serum sample at baseline (enrollment). Participants who tested positive for chlamydia, gonorrhea, or

syphilis were made aware of their STI results and were provided treatment. Counselling regarding safe sexual practice was provided to all the study participants at each clinical visit, regardless of age, as mandated by South African guidelines, including providing condoms free of charge.

During each of the three follow up visits, a pregnancy test using a urine sample and a rapid HIV test were performed. As stated, any participant with a positive pregnancy test was referred to care and was removed from the EVRI trial. Participants with a positive rapid HIV test were retested with two different confirmatory tests and those that were confirmed positive (n=3) were referred to care but remained on the trial, but were removed from HPV related analysis as HIV is known to influence HPV natural history.[21]

External genitalia were examined at enrollment and Month 7 for any signs of skin pigmentation, lesions, discharge and nodules. This was followed by a speculum examination of the vagina and a digital vaginal examination. Samples obtained from the vulva/labia and endo/ectocervical specimens were collected for HPV detection at enrollment and Month 7. Specimens for HPV analysis were obtained using a prewetted Dacron Cotton Swab placed in an STM collection vial (manufactured by Digene Corporation, Maryland) and were stored at 4^0C before testing. If the Pap smear cytology (collected using SurePath method) results were greater than atypical squamous cells of undetermined significance (ASCUS)/low-grade squamous intraepithelial lesions (LSIL), the participant was referred to a local clinic to perform a repeat Pap smear test and clinical management was indicated. Participants with Pap cytology results that showed high-grade squamous intraepithelial lesions (HSIL) were referred to the study gynecologist for colposcopy and clinical management.

Laboratory Analyses

Gonorrhea and chlamydia were detected using the Amplex CT/NG real- time detection method (Seegene, South Korea).[10] Syphilis was detected using Captia Syphilis (*Treponema pallidum*)-G assay (Trinity Biotech, Jamestown, NY). Specimens were reported positive only if reconfirmed

positive by a repeat test using the same assay.[22] HSV-2 antibody status was assessed using the Captia HSV-2 type specific IgG enzyme-linked immunoassay (EIA, Trinity Biotech). HIV status was assessed using the rapid testing method, Determine HIV-1/2 Ag/Ab combo immunoassay (Alere Healthcare, Waltham, MA). Specimens that were HIV-positive in the rapid test underwent two confirmatory tests with the Abbott HIV Ag/Ab combo (Abbott, Wiesbaden, Germany) and the BioMerieux VIDAS HIV DUO assay (BioMerieux Inc., Durham, NC).

HPV analysis was performed on the DNA that was extracted from the cervical cell specimens using the Qiagen Media Kit and amplified by polymerase chain reaction (PCR) with the PGMY09/11 L1 consensus primer system and AmpliTaq Gold polymerase (Perkin-Elmer, Norwalk, CT). HPV genotyping was performed on all specimens, regardless of PCR results, using the Linear Array HPV Genotyping Test (Roche Diagnostics, Indianapolis, IN). This test detects 37 HPV genotypes including 13 high-risk HPV genotypes (HR-HPV): 16,18,31,33,35,39,45,51,52,56,58,59,68 and 24 low-risk HPV genotypes (LR-HPV): 6,11,26,40,42,53-55,61,62,64,66,67,69-73,81-84,89,IS39.[23] The Linear array method, however, cannot detect HPV 52 co-infection in the presence of HPV genotypes 33, 35 or 58, so some of the HPV 52 co-infections may have gone undetected in this trial.

Statistical Analysis

Among the 402 women enrolled, three participants with false negative results for HIV, five participants with inadequate samples for HPV detection, and six participants who were β-globin and HPV DNA negative at enrollment were removed from the analysis. Specimens from the remaining 388 participants at enrollment were used in the analysis, as described in Figure 2. Statistical analyses was performed using SAS ® 9.4 software (SAS Institute Inc., Cary, NC, USA). A probability of less than 0.05 was considered statistically significant.

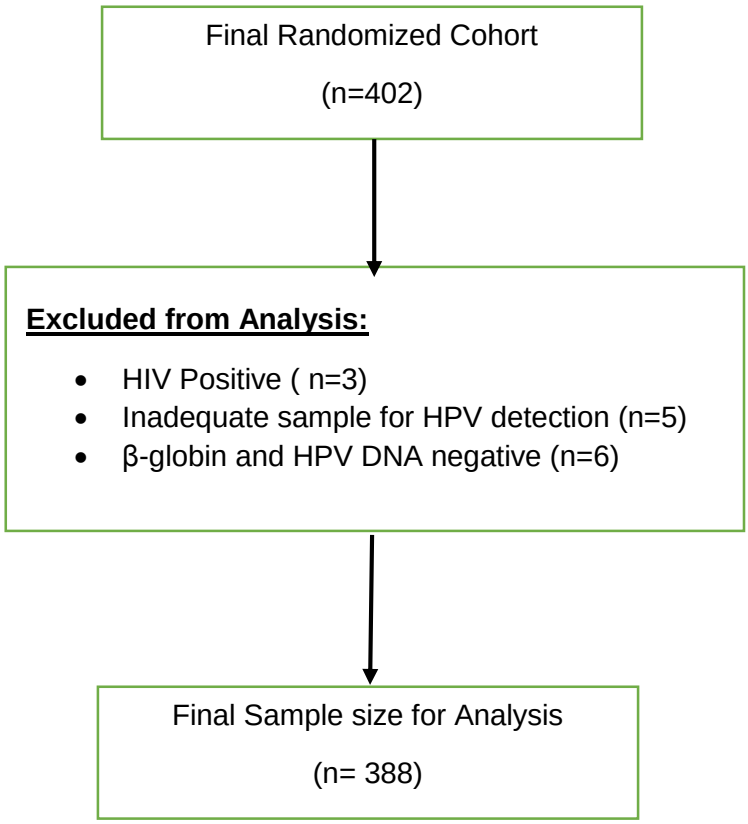


Figure 2. The EVRI Study: Final Sample Size Flowchart for Analysis

At enrollment women were tested for five STIs: HPV, chlamydia, gonorrhea, HSV-2 and syphilis and were categorized into three categories: single infection, STI co-infection, and no infection. Single infection was defined as having any one of the five STIs, co-infection was defined as having a combination of two or more of these STIs, and no infection was defined as being negative for all STIs.

Enrollment demographic and sexual health characteristics were compared between the three groups using Wilcoxon rank-sum test for continuous variables, and Fisher's exact test or chi-squared test for categorical variables. HPV infection was further classified as having any HPV genotype or HR-HPV genotype. Demographic variables were chosen based on associations with

STIs shown in previous studies. [9, 10]. Unadjusted and adjusted odds ratios assessing factors associated with STI co-infection were calculated using a polytomous logistic regression model comparing the three groups of women: STI negative, single STI infection and STI co-infections. Odds ratio (OR) and 95% confidence intervals (95% CI) were reported as the measure of association. A multivariate model was created using a backward selection procedure with a p-value of 0.20 to obtain the most parsimonious model. To determine the presence of effect modification, interactions were tested between the main effect variable and the covariates included in the model. Finally, to test whether our final model provided a good fit to the data, a Goodness-of-Fit test was performed.

Results

Demographic Characteristics (Any HPV)

When HPV was categorized as “any HPV” infection, 12% (n=48) women had no STIs, 34% (n=131) were infected with at least one and 54% (n=209) women had two or more STI co-infections (Table 1a). The median age of participants with “no infection” and participants with co-infections was 20 years (range, 16-24) whereas it was 21 years for the single infection group (range, 16-24). Education level was significantly different (p=0.02) between the three groups; women with STI co-infections had a larger proportion reporting less than 12th grade education level. Depo-Provera (23%-29%) and condoms (22%-27%) were the common birth control methods that were used and there were no significant differences between the three groups. The median number of life-time male sexual partners significantly differed between the groups (p=0.007) with the STI co-infection group having a larger number of partners compared to the no infection and single infection groups. Women who had STI co-infections were marginally (p=0.10) more likely to have abnormal cervical cytology (14%) compared to women who had no infection (4%) or had single infection (10%).

Table 1a. Demographic and sexual health characteristics of women in the EVRI Trial comparing women with and without STIs (Any HPV, Chlamydia, Gonorrhea, Syphilis, or HSV-2)

	No Infection (n=48)	Single Infection (n=131)	Co-infection (n=209)	P-value[a]
	N (%)	N (%)	N (%)	
Median age in years (range)	20(16-24)	21(16-24)	20(16-24)	0.24
16-18	12 (25.0)	22(16.7)	48(22.9)	
19-21	24(50.0)	64(48.8)	103(49.2)	
22-24	12(25.0)	45(34.3)	58(27.7)	
Marital status				0.31
Single/Widowed	44(91.6)	125(95.4)	202(96.6)	
Married/Living Together	4(8.3)	6(4.5)	7(3.3)	
Education				**0.02**
≤ Grade 7	6(12.5)	3(2.2)	10(4.7)	
Grade 8-12	21(43.7)	65(49.6)	126(60.2)	
Passed Grade 12	11(22.9)	33(25.1)	34(16.2)	
Some college/tech	10(20.8)	30(22.9)	39(18.6)	
Median age of first vaginal sex (range)	16(13-19)	17(13-21)	17(1-21)	0.33
Currently using birth control[b]	21(43.7)	62(47.3)	101(48.3)	0.81
Current birth control use				
Oral contraceptives	1(2.0)	6(4.5)	7(3.3)	0.69
IUD/loop/coil	0(0.0)	1(0.7)	1(0.4)	0.81
Depo Provera	11(22.9)	38(29.0)	50(23.9)	0.52
Condoms	13(27.0)	29(22.1)	50(23.9)	0.78
Ever been pregnant	21(43.7)	67(51.1)	104(49.7)	0.67

Table 1a. (Continued)

	No Infection (n=48)	Single Infection (n=131)	Co-infection (n=209)	P-value[a]
	N (%)	N (%)	N (%)	
Lifetime no. of male sexual partners[c]; median (range)	2 (1-17)	2(1-22)	3(1-24)	**0.0074**
1	10(33.3)	18(18.1)	27(16.3)	
2	11(36.6)	32(32.3)	44(26.6)	
3+	9(30.0)	49(49.4)	94(56.9)	
Number of sexual partners in the past 6 months[d]; median (range)	1(0-3)	1(0-5)	1(0-5)	0.48
0	2(8.3)	6(8.1)	8(6.3)	
1	19(79.1)	56(75.6)	91(72.2)	
2	2(8.3)	8(10.8)	21(16.6)	
3+	1(4.1)	4(5.4)	6(4.7)	
Ever received money/drugs/ presents for sex	3(6.2)	5(3.8)	5(2.3)	0.38
Cervical Cytology[e]				0.10
Normal	46(95.8)	118(90.0)	179(85.6)	
Abnormal	2(4.1)	13(9.9)	30(14.3)	

Bold text denotes statistically significant findings

[a] P-value calculated as based on Pearson-Chi Square test for categorical variables and Wilcoxon Mann-Whitney test for continuous variables

[b] sample size reduced due to missing values (n=134) ;

[c,d] sample size reduced due to missing values (n=94) and (n=164) respectively ;

[e] abnormal cervical cytology= High grade Squamous intraepithelial lesion(HSIL) or Low grade Squamous intraepithelial lesion(LSIL); normal cervical cytology= negative pap test or atypical cells of undetermined significance (ASCUS)

Demographic Characteristics (HR-HPV)

The median age of participants among the single infection and co-infection groups was 20 years (range, 16-24) whereas it was 20.5 years for the no infection group (range, 16-24) (Table 1b). The majority of the participants in all three groups were either single or widowed and the majority

of them had not passed grade 12. Presence of abnormal cervical cytology did statistically significantly (p=0.02) differ between the three groups, with women who had STI co-infections more likely to have abnormal cervical cytology (15%), compared with the single infection (11%) and no infection (3%) groups. However, there was no other statistically significant difference in the demographic and sexual behavior between the three groups of women.

Table 1b. Demographic and sexual health characteristics of women in the EVRI Trial comparing women with and without STIs (HR- HPV, Chlamydia, Gonorrhea, Syphilis, or HSV-2)

	No Infection (n=68)	Single Infection (n=138)	Co-infection (n=182)	P-value[a]
	N (%)	N (%)	N (%)	
Median age in years (range)	20.5(16-24)	20(16-24)	20(16-24)	0.58
16-18	15(22.0)	26(18.8)	41(22.5)	
19-21	30(44.1)	69(50.0)	92(50.5)	
22-24	23(33.8)	43(31.1)	49(26.9)	
Marital status				0.37
Single/Widowed	63(92.6)	132(95.6)	176(96.7)	
Married/Living Together	5(7.3)	6(4.3)	6(3.3)	
Education				0.11
≤ Grade 7	7(10.2)	5(3.6)	7(3.8)	
Grade 8-12	28(41.1)	75(54.3)	109(59.8)	
Passed Grade 12	16(23.5)	30(21.7)	32(17.5)	
Some college/tech	17(25.0)	28(20.2)	34(18.6)	
Median age of first vaginal sex (range)	16(13-19)	17(13-21)	17(1-21)	0.94
Currently using birth control[b]	30(44.1)	67(48.5)	87(47.8)	0.95
Current birth control use				
Oral contraceptives	1(1.4)	7(5.0)	6(3.3)	0.40

Table 1b. (Continued)

	No Infection (n=68)	Single Infection (n=138)	Co-infection (n=182)	P-value[a]
	N (%)	N (%)	N (%)	
IUD/loop/coil	0(0.0)	2(1.4)	0(0.0)	0.16
Depo Provera	19(27.9)	37(26.8)	43(23.6)	0.71
Condoms	15(22.0)	32(23.1)	45(24.7)	0.89
Ever been pregnant	31(45.5)	71(51.4)	90(49.4)	0.73
Lifetime no. of male sexual partners[c]; median (range)	2 (1-22)	2.5(1-21)	3(1-24)	0.09
1	11(24.4)	21(19.8)	23(16.0)	
2	16(35.5)	32(30.1)	39(27.2)	
3+	18(40.0)	53(50.0)	81(56.6)	
Number of sexual partners in the past 6 months[d]; median (range)	1(0-3)	1(0-5)	1(0-5)	0.32
0	2(5.8)	8(9.6)	6(5.6)	
1	28(82.3)	61(73.4)	77(71.9)	
2	2(5.8)	11(13.2)	18(16.8)	
3+	2(5.8)	3(3.6)	6(5.6)	
Ever received money/drugs/ presents for sex	4(5.8)	4(2.9)	5(2.7)	0.44
Cervical Cytology[e]				**0.02**
Normal	66(97.0)	123(89.1)	154(84.6)	
Abnormal	2(2.9)	15(10.8)	28(15.3)	

Bold text denotes statistically significant findings

[a] P-value calculated as based on Pearson-Chi Square test for categorical variables and Wilcoxon Mann-Whitney test for continuous variables

[b] sample size reduced due to missing values (n=134) ;

[c,d] sample size reduced due to missing values (n=94) and (n=164) respectively ;

[e] abnormal cervical cytology= High grade Squamous intraepithelial lesion(HSIL) or Low grade Squamous intraepithelial lesion(LSIL); normal cervical cytology= negative pap test or atypical cells of undetermined significance (ASCUS)

Prevalence of STIs (Any HPV)

Prevalence of STI co-infections was high in the women enrolled in the EVRI Trial (Table 2). Among women with only a single infection, the prevalence of HPV (67%, n=88) was the highest (Figure 3a), followed by the prevalence of HSV-2 (19%, n=25), chlamydia (CT) (11%, n=14), and syphilis (SY) (3%, n=4). Gonorrhea (GN) was not seen as an isolated single infection in any enrolled women. The most common STI co-infection pattern was HPV-HSV (32%, n=67), followed by HPV-CT (17%, n=35); for three concurrent STI co-infections it was HPV-HSV-CT (16%, n=34) (Figure 3b). The most common four concurrent STI co-infections was HPV-CT-GN-HSV (7%, n=15) and about 1% (n=2) of enrolled women had all five STIs (HPV-CT-GN-SY-HSV) assessed.

Table 2: STI co-infection patterns of women in the EVRI Trial (Any HPV/ HR- HPV, Chlamydia, Gonorrhea, Syphilis, or HSV-2)

Nature of infections	Any HPV % (n)	HR-HPV %(n)
No infection at all	12.3 (48)	17.5 (68)
Single Infection		
HPV only	22.6 (88)	17.5 (68)
Chlamydia only	3.6 (14)	5.4 (21)
Gonorrhea only	0.0 (0)	0.0 (0)
Syphilis only	1.0 (4)	1.0 (4)
HSV only	6.4 (25)	11.5 (45)
2 Co-infections		
HPV + Chlamydia	9.0 (35)	7.2 (28)
HPV + Gonorrhea	1.2 (5)	1.2 (5)
HPV + Syphilis	0.5 (2)	0.5 (2)
HPV + HSV	17.2 (67)	12.1 (47)
Chlamydia + Gonorrhea	0.5 (2)	1.0 (4)
Chlamydia + Syphilis	0.0 (0)	0.0 (0)
Chlamydia + HSV	3.3 (13)	4.1 (16)
Gonorrhea + Syphilis	0.0 (0)	0.0 (0)
Gonorrhea + HSV	0.8 (3)	1.0 (4)
Syphilis + HSV	0.0 (0)	1.0 (4)
3 Co-infections		
HPV + Chlamydia+ Gonorrhea	2.3 (9)	1.8 (7)
HPV + Chlamydia + Syphilis	0.5 (2)	0.5 (2)

Table 2. (Continued)

Nature of infections	Any HPV % (n)	HR-HPV %(n)
HPV + Chlamydia + HSV	8.7 (34)	7.9 (31)
HPV + Gonorrhea + Syphilis	0.0 (0)	0.0 (0)
HPV + Gonorrhea + HSV	1.2 (5)	1.0 (4)
HPV + HSV + Syphilis	2.8 (11)	1.8 (7)
Chlamydia + Gonorrhea + Syphilis	0.0 (0)	0.0 (0)
Chlamydia + Gonorrhea + HSV	0.5 (2)	0.8 (3)
Gonorrhea + Syphilis + HSV	0.0 (0)	0.0 (0)
Chlamydia + Syphilis + HSV	0.0 (0)	0.0 (0)
4 Co-infections		
HPV + Chlamydia+ Gonorrhea + Syphilis	0.0 (0)	0.0 (0)
HPV + Chlamydia+ Gonorrhea + HSV	3.8 (15)	3.6 (14)
HPV + Chlamydia+ HSV + Syphilis	0.5 (2)	0.5 (2)
HPV + Gonorrhea + HSV + Syphilis	0.0 (0)	0.0 (0)
Chlamydia + Gonorrhea + Syphilis + HSV	0.0 (0)	0.2 (1)
5 Co-infections	0.5 (2)	0.2 (1)

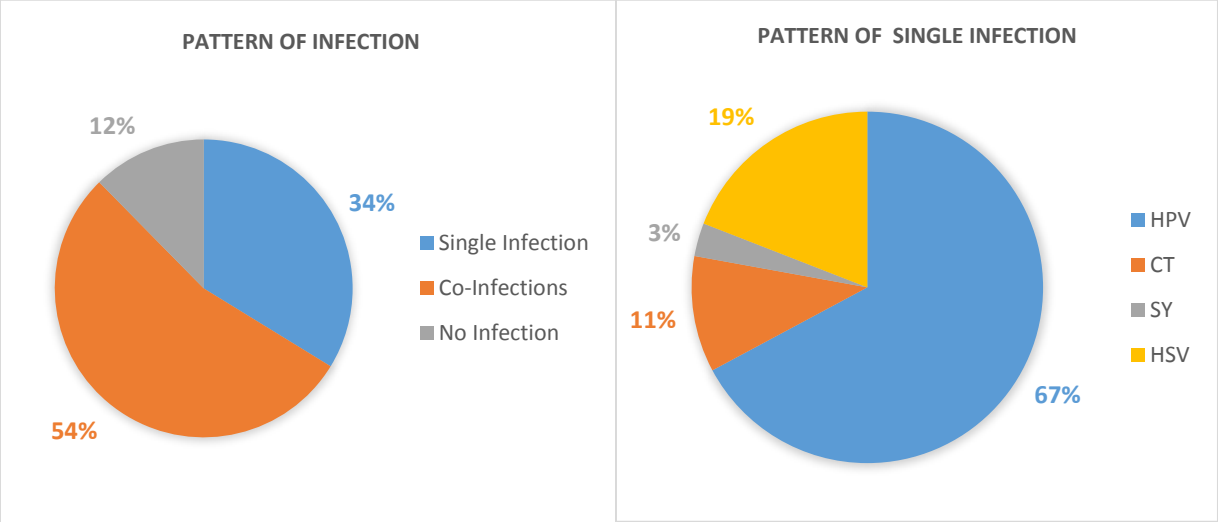


Figure 3a: STI infection patterns of women in the EVRI Trial with at least 1 STI (HPV=Any HPV, CT=Chlamydia, GN=Gonorrhea, SY=Syphilis, or HSV=Herpes Simplex -2)

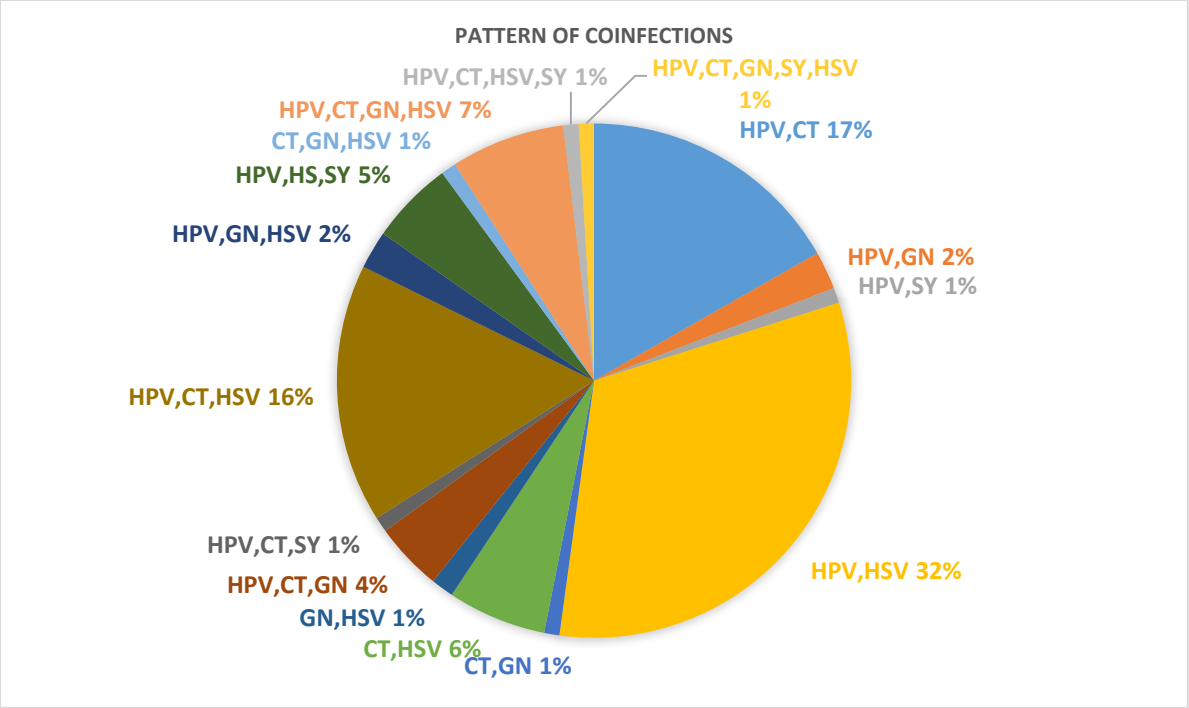
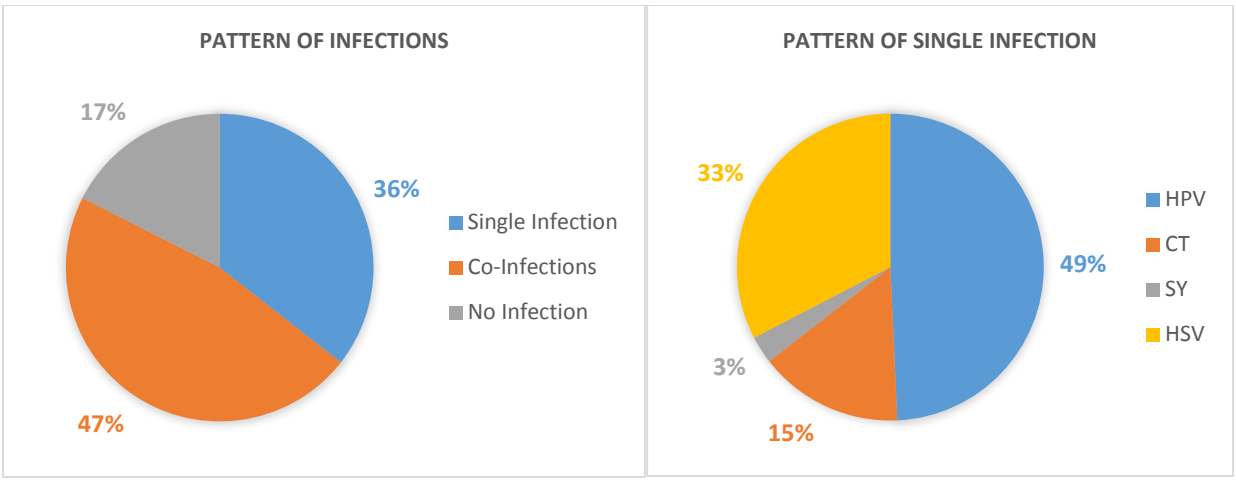


Figure 3b: STI co-infection patterns of women in the EVRI Trial with at least 1 STI (HPV=Any HPV, CT=Chlamydia, GN=Gonorrhea, SY=Syphilis, or HSV=Herpes Simplex -2)

Prevalence of STIs (HR-HPV)

Prevalence of co-infection was high among the women enrolled in the EVRI Trial when HPV infection was also restricted to HR-HPVs (Table 2). Among women with only a single STI, high-risk HPV had the highest prevalence at 49% (n=68) (Figure 3c), followed by HSV-2 (33%, n=45), CT infection (15%, n=21), and SY (3%, n=4). GN was not seen as an isolated single infection in any enrolled women. The most common co-infection pattern seen was High-risk HPV-HSV (26%, n=47), followed by High-risk HPV-CT (15%, n=28) looking at two STI co-infection followed by a co-infection with High-risk HPV-HSV-CT (17%, n=31) for a three STI co-infection pattern. The most common four concurrent STI co-infection pattern was seen for High-risk HPV-CT-GN-HSV (8%, n=14) and again about 1% (n=1) of enrolled women had all five STIs (High-risk HPV-CT-GN-SY-HSV) assessed.



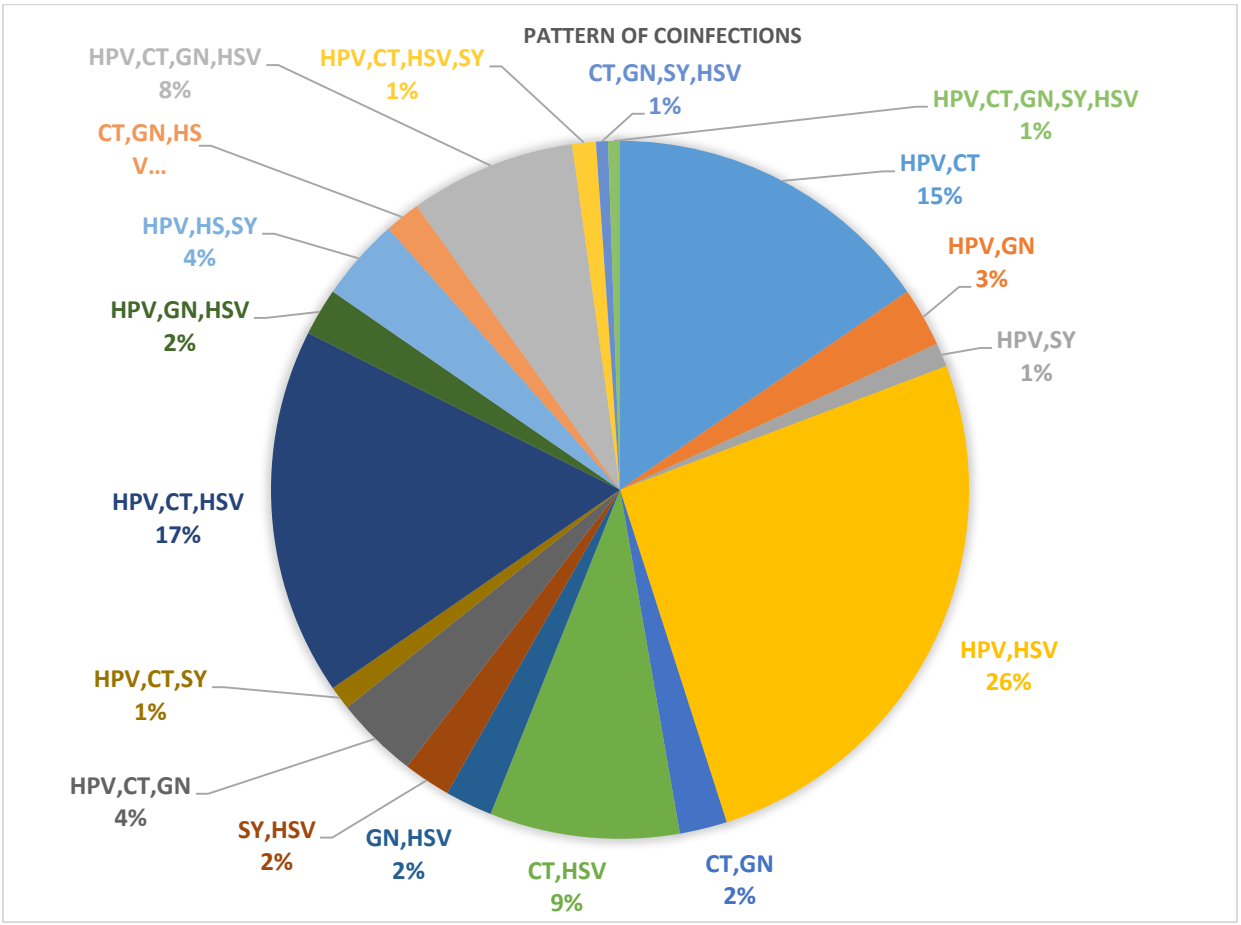


Figure 3c: STI co-infection patterns of women in the EVRI Trial with at least 1 STI (HPV=HR-HPV, CT=Chlamydia, GN=Gonorrhea, SY=Syphilis, or HSV=Herpes Simplex -2)

Factors Associated with STIs (Any HPV)

An unadjusted polytomous logistic regression analysis was used to identify the factors associated with single STI infection and STI co-infection compared to women with no STIs (Table 3a). The odds of a single infection and co-infection compared to no infection were non-significantly lower for ages 19-21 (OR 0.71, 95%CI: 0.32-1.56 for single STI infection and OR 0.88, 95% CI: 0.41-1.90 for STI co-infection) and ages 16-18 (OR 0.48, 95%CI: 0.18-1.26 for single STI infection and OR 0.82, 95% CI: 0.34-2.00 for STI co-infection) when compared to ages 22-24. The odds of having a single STI infection (OR 0.52, 95% CI: 0.14-1.95) or STI co-infection (OR 0.38, 95%CI: 0.10-1.35) was non-significantly lower in women who were married or living with partners compared to women who were single or widowed. Higher education was associated with lower odds of having an STI compared to no infection. The odds of single STI infection compared to no infection was significantly lower for women who had education level of grade 7 compared to women who had some college or technical education (unadjusted OR (uOR) 0.16, 95% CI: 0.03-0.79). The odds of a single STI infection compared to no infection were significantly higher (uOR 3.02, 95% CI: 1.05-8.64) for women with three or more lifetime partners compared to one life time partner. Similarly, the odds of a STI co-infection compared to no infection were significantly higher for women with three or more lifetime partners compared to one life time partner (uOR 3.86, 95% CI: 1.42-10.48). When we performed the backward selection model, the odds of STI co-infection was significantly higher in women who had three or more than three lifetime number of male sexual partners compared to women who had one lifetime male sexual partners (adjusted OR (aOR) 3.68, 95% CI: 1.14-11.90).

Table 3a. Factors associated with single STI (Any HPV, Chlamydia, Gonorrhea, Syphilis, or HSV-2) infection and STI co-infections compared to women with no infection among women in the EVRI Trial

	Single Infection (n=131)		Co-infections (n=209)	
	uOR[a] (95% CI)	aOR[b] (95% CI)	uOR[a] (95% CI)	aOR[b] (95% CI)
Age in years				
22-24	1.00(Ref)		1.00(Ref)	
19-21	0.71(0.32-1.56)	1.43(0.46-4.40)	0.88(0.41-1.90)	1.47(0.49-4.37)
16-18	0.48(0.18-1.26)	0.51(0.13-1.98)	0.82(0.34-2.00)	1.05(0.30-3.58)
Marital status				
Single/Widowed	1.00(Ref)		1.00(Ref)	
Married/Living Together	0.52(0.14-1.95)		0.38(0.10-1.35)	
Education				
Some college/tech	1.00(Ref)		1.00(Ref)	
≤ Grade 7	**0.16(0.03-0.79)**		0.42(0.12-1.45)	
Grade 8-12	1.03(0.43-2.45)		1.53(0.66-3.54)	
Passed Grade 12	1.00(0.37-2.68)		0.79(0.30-2.09)	
Currently using Birth Control[c]				
Yes	1.00(Ref)		1.00(Ref)	
No	1.25(0.47-3.32)		1.09(0.43-2.79)	
Ever been Pregnant				
Never	1.00(Ref)		1.00(Ref)	
Ever	0.74(0.38-1.44)		0.78(0.41-1.47)	
Lifetime no. of male sexual partners[d]				
1	1.00(Ref)	1.00(Ref)	1.00(Ref)	1.00(Ref)
2	1.61(0.57-4.54)	2.18(0.59-7.95)	1.48(0.55-3.95)	1.98(0.60-6.50)
3+	**3.02(1.05-8.64)**	3.28(0.92-11.67)	**3.86(1.42-10.48)**	**3.68(1.14-11.90)**
Number of sexual partners in the past 6 months[e]				

Table 3a. (Continued)

	Single Infection (n=131)		Co-infections (n=209)	
	uOR[a] (95% CI)	aOR[b] (95% CI)	uOR[a] (95% CI)	aOR[b] (95% CI)
0	1.00(Ref)		1.00(Ref)	
1	0.98(0.18-5.28)		1.19(0.23-6.09)	
2+	1.33(0.14-12.36)		2.25(0.31-15.90)	
Cervical Cytology[f]				
Normal	1.00(Ref)		1.00(Ref)	
Abnormal	2.53(0.55-11.64)	2.77(0.32-23.93)	3.85(0.88-16.69)	3.74(0.46-30.10)

Abbreviations: HPV: human papillomavirus; 95% CI: 95% Confidence Interval; ref: reference group

Bold text denotes statistically significant findings

[a] uOR: unadjusted Odds ratio; [b]aOR: adjusted odds Ratio;

[c] sample size reduced due to missing values (n=134) ;

[d,e] sample size reduced due to missing values (n=94) and (n=164) respectively ;

[f] abnormal cervical cytology= High grade Squamous intraepithelial lesion(HSIL) or Low grade Squamous intraepithelial lesion(LSIL); normal cervical cytology= negative pap test or atypical cells of undetermined significance (ASCUS)

[g] uOR was estimated by multinomial logistics regression model/link=glogit function;

[h] aOR was estimated by multinomial logistics regression model using /link=glogit function and using a backward selection procedure with a stay p-value of 0.20. Variables age, lifetime number of male sexual partners and cervical cytology were forced in the adjusted model.

Factors Associated with STIs (HR-HPV)

When the analysis was restricted to women who were infected with high risk HPV (HR-HPV) types, no independent risk factor was statistically significantly associated with the risk of single STI infection compared to no infection in the unadjusted model. However, the odds of STI co-infection was significantly higher in women who had abnormal cervical cytology, categorized as presence of High–grade and Low-grade squamous intraepithelial lesion (HSIL/LSIL), compared to women who had no infection (uOR 6.00, 95% CI: 1.38-25.91) (Table 3b). When we performed the backward selection model, the odds of STI co-infection compared to no infection was significantly higher in women who had three or more than three lifetime number of male sexual partners compared to women who had one lifetime male sexual partners (aOR 3.10, 95% CI: 1.02-9.34).

Table 3b. Factors associated with single STI (HR-HPV, Chlamydia, Gonorrhea, Syphilis, or HSV-2) infection and STI co-infections compared to women with no infection among women in the EVRI Trial

	Single Infection (n=138)		Co-infections (n=182)	
	uOR[a] (95% CI)	aOR[b] (95% CI)	uOR[a] (95% CI)	aOR[b] (95% CI)
Age in years				
22-24	1.00(Ref)		1.00(Ref)	
19-21	1.23(0.63-2.38)	1.36(0.53-3.51)	1.43(0.75-2.74)	1.56(0.61-3.97)
16-18	0.92(0.41-2.08)	0.69(0.20-2.30)	1.28(0.59-2.77)	1.50(0.49-4.63)
Marital status				
Single/Widowed	1.00(Ref)		1.00(Ref)	
Married/Living together	0.57(0.16-1.94)		0.43(0.12-1.45)	
Education				
Some college/tech	1.00(Ref)		1.00(Ref)	
≤ Grade 7	0.43(0.11-1.58)		0.50(0.15-1.65)	
Grade 8-12	1.62(0.77-3.41)		1.94(0.95-3.97)	
Passed Grade 12	1.13(0.48-2.67)		1.00(0.43-2.30)	
Currently using Birth Control[c]				
Yes	1.00(Ref)		1.00(Ref)	
No	0.89(0.40-1.97)		0.82(0.38-1.77)	
Ever been Pregnant				
Never	1.00(Ref)		1.00(Ref)	
Ever	0.79(0.44-1.41)		0.85(0.49-1.49)	
Lifetime no. of male sexual partners[d]				
1	1.00(Ref)	1.00(Ref)	1.00(Ref)	1.00(Ref)
2	1.04(0.40-2.69)	1.46(0.45-4.66)	1.16(0.46-2.93)	2.01(0.64-6.34)
3+	1.54(0.62-3.81)	1.99(0.65-6.06)	2.15(0.89-5.19)	**3.10(1.02-9.34)**
Number of sexual partners in the past 6 months[e]				

Table 3b. (Continued)

	Single Infection (n=138)		Co-infections (n=182)	
	uOR[a] (95% CI)	aOR[b] (95% CI)	uOR[a] (95% CI)	aOR[b] (95% CI)
0	1.00(Ref)		1.00(Ref)	
1	0.54(0.10-2.73)		0.91(0.17-4.81)	
2	0.87(0.13-5.89)		2.00(0.29-13.62)	
Cervical Cytology[f]				
Normal	1.00(Ref)		1.00(Ref)	
Abnormal	4.02(0.89-18.13)	4.51 (0.54-37.08)	**6.00(1.38-25.91)**	5.74(0.72-45.75)

Abbreviations: HPV: human papillomavirus; 95% CI: 95% Confidence Interval; ref: reference group

Bold text denotes statistically significant findings

[a] uOR: unadjusted Odds ratio; [b]aOR: adjusted odds Ratio;

[c] sample size reduced due to missing values (n=134) ;

[d,e] sample size reduced due to missing values (n=94) and (n=164) respectively ;

[f] abnormal cervical cytology= High grade Squamous intraepithelial lesion(HSIL) or Low grade Squamous intraepithelial lesion(LSIL); normal cervical cytology= negative pap test or atypical cells of undetermined significance (ASCUS)

[g] uOR was estimated by multinomial logistics regression model/link=glogit function;

[h] aOR was estimated by multinomial logistics regression model using /link=glogit function and using a backward selection procedure with a stay p-value of 0.20. Variables age, lifetime number of male sexual partners and cervical cytology were forced in the adjusted model.

Discussion

We have described the prevalence of HPV and other common STIs along with factors associated with single infection and coinfection among young South African women who participated in the EVRI Trial. STIs were common and the women with STIs were likely to have multiple co-infections. This high STI prevalence may have an alarming impact on the women's reproductive health and potential future impact on pregnancy birth outcomes. It highlights the need for an effective and coordinated intervention for HPV and other common STIs.

Overall prevalence of a single STI and STI co-infections are consistent with similar studies performed in the sub-Saharan African region. Our study showed an overall chlamydia prevalence of 33%. A study conducted in Mozambique showed that the overall prevalence of chlamydia infection was 8% [8] while another study among women in South Africa showed chlamydia prevalence of 19.5%.[16] In Uganda, prevalence of chlamydia in female adolescents was 4.5%.[24] Women with chlamydia infection were shown to be at high risk of acquiring gonorrhea and vice-versa.[16] Our study showed an overall syphilis prevalence of 6%. A study conducted among women in rural sub-Saharan Africa showed that prevalence of syphilis was 12% [8] while another study reported prevalence of 7.5% [16] in South Africa. In a cohort of women from rural Tanzania, syphilis prevalence was reported to be 9.1%.[25] The high prevalence of bacterial STIs is not consistently seen across countries but rather seems to be country specific.[10] One of the reasons for this might be the difference in the sensitivity of the assays used for detection.[16] Overall prevalence of HSV-2 antibodies were high in our study at 46%. Prevalence of HSV-2 among women in rural sub- Saharan Africa was 83% in one study. [8] Similar studies in South Africa reported an HSV-2 prevalence estimate between 40-70%.[26] The high prevalence of HSV-

2 is particularly important as it has been shown that HSV-2 increases the susceptibility of HIV transmission and HSV-2 seropositivity has been attributed as a marker of sexual health behavior among adolescents.[17, 18]

The overall prevalence of HPV in this study was high at 71% as previously reported from the EVRI trial.[10] In one study cervical HPV prevalence was estimated at 22% among women in Africa [7] and among women in rural sub Saharan Africa, another study reported an HPV prevalence of 40%.[8] A comprehensive meta-analysis of HPV prevalence from 71 studies from 23 African countries showed that there is regional variation with Southern Africa having the highest prevalence (57%) followed by Eastern Africa (42%), Western Africa (29%) and Northern Africa (13%).[27] HPV prevalence in our study might have been higher compared to these studies because of the age of women enrolled in the EVRI trial and the study site for trial being chosen based on the clinical data that showed high prevalence of HIV in the area. It has been shown consistently across studies that HPV prevalence decreases with increasing age from a peak prevalence in younger women (<=25 years of age).[28] HPV prevalence in our study is comparable with data seen among female sex workers and HIV-positive women in Africa, where the prevalence ranged from 39-71%.[29] High prevalence of HPV in these studies of young women suggests that vaccination against HPV needs to be started early in HPV high-risk communities. Co-infection with other STIs like chlamydia and HSV-2 are significant risk factors for increased HPV infection.[30] High prevalence of chlamydial infection in these study populations has a positive association with HR-HPV persistence and regular screening and management of STIs are a potential prevention strategies to reduce HR-HPV persistence and related adverse health outcomes among women.[6]

We observed a strong association between the lifetime number of male sexual partners and risk of an STI or STI co-infections. The odds of a single STI and STI co-infection compared to no infection were significantly higher for women with three or more lifetime partners compared to one

life time partner. These findings are consistent with a study carried out in three different African countries, South Africa, Zambia and Tanzania, that showed that having two or more male sexual partners, being unmarried and having higher education were independent risk factors for getting a STI.[16] A longitudinal study among young adolescent women showed that having a new sexual partner results in a 10-fold increased risk for getting HPV. [31] High-risk sexual behaviors like multiple sexual partners and non-use of condoms are usually associated with increased risk of STIs. Behavioral interventions have been promoted as an essential tool to combat the transmission of STIs worldwide. We found that higher education increased the risk of an STI infection. The formation of “sexual partnerships” play a key role in the transmission dynamics of STIs and places that offer higher education (college, universities) are the most common places for forming these partnerships.[4] Similarly, when restricted to analysis among high-risk HPV positive women, the odds of STI co-infection compared to no infection was significantly higher for women with abnormal cytology compared to normal cytology. Although the temporality of this association cannot be explained, it has been shown that early sexual activity and the presence of STIs accelerates the process of cervical maturation, which in the presence of a persistent HPV infection, induces genetic alteration in the epithelium that can lead to high-grade squamous intraepithelial lesions.[31]

There are several strengths and limitations to the current study which need to be considered in interpreting the data and drawing conclusions. A major strength of our study is the large sample size that allowed us to quantify STI prevalence and describe co-infections with STIs. Syndromic management is the treatment of choice for many STIs and our prevalence data show it remains ineffective among young adolescents in developing countries, as many of the infections we detected were asymptomatic.

Since the EVRI baseline data were obtained indirectly using tablet- based questionnaires, data on sexual and behavioral characteristics might have been affected by social-desirability bias. This

could potentially introduce misclassification into our study due to underestimation of the reported number of lifetime male sexual partners which might have affected the capability to assess associations. Also, the tablet-based questionnaire was a source of initial confusion in the trial as 116 participants reported not having had vaginal intercourse during the eligibility screening. The study site for the EVRI trial was chosen based on the clinical data that showed high prevalence of HIV in the area. Therefore, our study findings might not be generalizable to all South African women. Our study also is likely to be underpowered for some of the tested associations. There might have been suggestive associations that might have been significant with a larger sample. STIs like HPV were based on DNA detection whereas other STIs like HSV-2 were based on serology that might not be a reflection of the current infection status. Lastly, the prevalence of other important STIs, like trichomonas was not measured in the EVRI trial.

The data from the EVRI trial clearly demonstrate that behavioral risk factors play an important role in STI prevalence. These results strengthen the recommendation that STI screening and treatment needs to be a component of multiple intervention strategies among high-risk women, such as those residing in communities with high STI prevalence. For HPV infections, recent developments in vaccines offers a lot of hope, but the challenge remains on the affordability and availability of these in rural areas where HPV is prevalent. A transdisciplinary prevention approach involving vaccines, effective treatments, behavioral interventions, counseling, proper education, elaborate community participation and effective health care policy is needed to have a measurable impact on highly prevalent STIs in these population of young women.

References

1. Sturm, A.W., et al., *Pregnant women as a reservoir of undetected sexually transmitted diseases in rural South Africa: implications for disease control.* Am J Public Health, 1998. **88**(8): p. 1243-5.
2. World Health Organization. *Global incidence and prevalence of selected curable sexually transmitted infections* 2008 [cited 2015 18 Feb].
3. Moodley, P. and A.W. Sturm, *Sexually transmitted infections, adverse pregnancy outcome and neonatal infection.* Semin Neonatol, 2000. **5**(3): p. 255-69.
4. Zuma, K., et al., *Risk factors of sexually transmitted infections among migrant and non-migrant sexual partnerships from rural South Africa.* Epidemiol Infect, 2005. **133**(3): p. 421-8.
5. Munoz, N., et al., *Incidence, duration, and determinants of cervical human papillomavirus infection in a cohort of Colombian women with normal cytological results.* J Infect Dis, 2004. **190**(12): p. 2077-87.
6. Vielot, N., et al., *The Role of Chlamydia trachomatis in High-Risk Human Papillomavirus Persistence Among Female Sex Workers in Nairobi, Kenya.* Sex Transm Dis, 2015. **42**(6): p. 305-11.
7. de Sanjose, S., et al., *Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis.* Lancet Infect Dis, 2007. **7**(7): p. 453-9.

8. Menendez, C., et al., *Prevalence and risk factors of sexually transmitted infections and cervical neoplasia in women from a rural area of southern Mozambique.* Infect Dis Obstet Gynecol, 2010. **2010**.
9. Sudenga, S.L. and S. Shrestha, *Key considerations and current perspectives of epidemiological studies on human papillomavirus persistence, the intermediate phenotype to cervical cancer.* Int J Infect Dis, 2013. **17**(4): p. e216-20.
10. Giuliano, A.R., et al., *High HIV, HPV, and STI prevalence among young Western Cape, South African women: EVRI HIV prevention preparedness trial.* J Acquir Immune Defic Syndr, 2015. **68**(2): p. 227-35.
11. Joura, E.A., et al., *Attribution of 12 high-risk human papillomavirus genotypes to infection and cervical disease.* Cancer Epidemiol Biomarkers Prev, 2014. **23**(10): p. 1997-2008.
12. Lewis, D.A., *HIV/sexually transmitted infection epidemiology, management and control in the IUSTI Africa region: focus on sub-Saharan Africa.* Sex Transm Infect, 2011. **87 Suppl 2**: p. ii10-13.
13. Centers for Disease Control & Prevention (CDC), *Sexually Trasmitted Disease Surveillance 2013*. 2014.
14. Centers for Disease Control & Prevention (CDC), *Prevalence of Chlamydia trachomatis genital infection among person aged 14-39 years- United States, 2007-2009*, in *MMWR. Moridity and Mortality Weekly Reports*. 2014.
15. Parish, W.L., et al., *Population-based study of chlamydial infection in China: a hidden epidemic.* JAMA, 2003. **289**(10): p. 1265-73.
16. Kapiga, S., et al., *Risk factors for incidence of sexually transmitted infections among women in South Africa, Tanzania, and Zambia: results from HPTN 055 study.* Sex Transm Dis, 2009. **36**(4): p. 199-206.

17. Obasi, A., et al., *Antibody to herpes simplex virus type 2 as a marker of sexual risk behavior in rural Tanzania.* J Infect Dis, 1999. **179**(1): p. 16-24.

18. Todd, J., et al., *Risk factors influencing HIV infection incidence in a rural African population: a nested case-control study.* J Infect Dis, 2006. **193**(3): p. 458-66.

19. Xu, F., et al., *Trends in herpes simplex virus type 1 and type 2 seroprevalence in the United States.* JAMA, 2006. **296**(8): p. 964-73.

20. Ward, H. and M. Ronn, *Contribution of sexually transmitted infections to the sexual transmission of HIV.* Curr Opin HIV AIDS, 2010. **5**(4): p. 305-10.

21. Minkoff, H., et al., *A longitudinal study of human papillomavirus carriage in human immunodeficiency virus-infected and human immunodeficiency virus-uninfected women.* Am J Obstet Gynecol, 1998. **178**(5): p. 982-6.

22. Halling, V.W., et al., *Clinical comparison of the Treponema pallidum CAPTIA syphilis-G enzyme immunoassay with the fluorescent treponemal antibody absorption immunoglobulin G assay for syphilis testing.* J Clin Microbiol, 1999. **37**(10): p. 3233-4.

23. Bouvard, V., et al., *A review of human carcinogens--Part B: biological agents.* Lancet Oncol, 2009. **10**(4): p. 321-2.

24. Rassjo, E.B., et al., *Prevalence of sexually transmitted infections among adolescents in Kampala, Uganda, and theoretical models for improving syndromic management.* J Adolesc Health, 2006. **38**(3): p. 213-21.

25. Todd, J., et al., *Risk factors for active syphilis and TPHA seroconversion in a rural African population.* Sex Transm Infect, 2001. **77**(1): p. 37-45.

26. De Baetselier, I., et al., *Prevalence and incidence estimation of HSV-2 by two IgG ELISA methods among South African women at high risk of HIV.* PLoS One, 2015. **10**(3): p. e0120207.

27. Ogembo, R.K., et al., *Prevalence of human papillomavirus genotypes among African women with normal cervical cytology and neoplasia: a systematic review and meta-analysis.* PLoS One, 2015. **10**(4): p. e0122488.

28. Smith, J.S., et al., *Age-specific prevalence of infection with human papillomavirus in females: a global review.* J Adolesc Health, 2008. **43**(4 Suppl): p. S5-25, S25 e1-41.

29. LaMontagne, D.S., et al., *Human papillomavirus vaccine delivery strategies that achieved high coverage in low- and middle-income countries.* Bull World Health Organ, 2011. **89**(11): p. 821-830B.

30. Abbai, N.S., T. Reddy, and G. Ramjee, *Prevalent bacterial vaginosis infection - a risk factor for incident sexually transmitted infections in women in Durban, South Africa.* Int J STD AIDS, 2015.

31. Moscicki, A.B., et al., *Risks for incident human papillomavirus infection and low-grade squamous intraepithelial lesion development in young females.* JAMA, 2001. **285**(23): p. 2995-3002.

Appendix A: Eligibility Criteria

1.	Are you between 16-24 years of age?	**Yes**	No
2.	Have you ever had an abnormal Pap smear ?	Yes	**No**
3.	Have you ever had vaginal intercourse?	**Yes**	No
4.	Are you currently pregnant?	Yes	**No**
5.	Are you currently breastfeeding?	Yes	**No**
6.	Have you ever been diagnosed with AIDS or HIV?	Yes	**No**
7.	Do you have an autoimmune disease for which you are taking steroids?	Yes	**No**
8.	Have you had a splenectomy?	Yes	**No**
9.	Are you currently taking part in an HIV prevention trial?	Yes	**No**
10.	Are you now or have you during past 6 months used IV drugs or TIK?	Yes	**No**
11.	Do you have any history of serious allergic reaction requiring Medical attention?	Yes	**No**
12.	Do you have a known allergy to Aluminum, Yeast or Benzonase?	Yes	**No**
13.	Have you ever received a marketed HPV vaccine?	Yes	**No**
14.	Are you willing to comply with 4 scheduled visits within the next 7 months?	**Yes**	No
15.	Do you agree to use effective contraception during sexual Intercourse (excluding the rhythm method or withdrawal) during the vaccination period?	**Yes**	No

Appendix B: IRB Approval Letter

RESEARCH INTEGRITY AND COMPLIANCE
Institutional Review Boards, FWA No. 00001669
12901 Bruce B. Downs Blvd., MDC035 • Tampa, FL 33612-4799
(813) 974-5638 • FAX (813) 974-7091

1/8/2016

Anna Giuliano, Ph.D.
H Lee Moffitt Cancer Center
12902 Magnolia Drive
Tampa, FL 33612

RE: **Expedited Approval for Continuing Review**
IRB#: CR4_Pro00005120
Title: Preparedness Study to Test the Future Efficacy of HPV Vaccine to Reduce HIV Infection - MCC# 16685

Study Approval Period: 2/7/2016 to 2/7/2017

Dear Dr. Giuliano:

On 1/7/2016, the Institutional Review Board (IRB) reviewed and **APPROVED** the above application and all documents contained within including those outlined below.

Approved Item(s):
Protocol Document(s):
Protocol (version 9, 07/24/2013) - clean

The waiver of informed consent process and the waiver of HIPAA authorization have been renewed.

The IRB determined that your study qualified for expedited review based on federal expedited category number(s):

(8) Continuing review of research previously approved by the convened IRB as follows: (a) where (i) the research is permanently closed to the enrollment of new subjects; (ii) all subjects

have completed all research-related interventions; and (iii) the research remains active only for long-term follow-up of subjects; or (b) where no subjects have been enrolled and no additional risks have been identified; or (c) where the remaining research activities are limited to data analysis.

Per CFR 45 Part 46, Subpart D, this research involving children was approved under the greater than minimal risk category 45 CFR 46.405: Research is greater than minimal risk with the prospect of direct benefit to the participant.

As the principal investigator of this study, it is your responsibility to conduct this study in accordance with USF HRPP policies and procedures and as approved by the USF IRB. Any changes to the approved research must be submitted to the IRB for review and approval by an amendment. Additionally, all unanticipated problems must be reported to the USF IRB within five (5) calendar days.

We appreciate your dedication to the ethical conduct of human subject research at the University of South Florida and your continued commitment to human research protections. If you have any questions regarding this matter, please call 813-974-5638.

Sincerely,

E. Verena Jorgensen, M.D., Chairperson
USF Institutional Review Board