Published in final edited form as:

J Acquir Immune Defic Syndr. 2015 February 1; 68(2): 227–235. doi:10.1097/QAI.0000000000000425.

High HIV, HPV, and STI prevalence among young Western Cape, South African women: EVRI HIV prevention preparedness trial

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Keywords

HIV; HPV; HPV vaccine; STI; clinical trial

Introduction

Despite years of study and interventions, HIV prevalence among women in sub-Saharan Africa remains persistently high and is highest among young women in South Africa.¹ In 2011, the prevalence of HIV was 29.5% among South African women ages 15-49 years attending their first antenatal care visit.² The underlying cause of this high HIV burden is likely due to a combination of factors, including high prevalence of HIV in the general population, early age at first sexual intercourse, multiple sexual partners, and co-infection with other sexual transmitted infections (STIs).³

In addition to a high HIV burden, women and men residing in southern African countries have among the highest burden of human papillomavirus (HPV) infection and related cancers worldwide.⁴⁻⁷ A growing literature suggests that, similar to HSV-2 and bacterial STIs, HPV infection may increase susceptibility to HIV. HPV was associated with a two- to three-fold increase in HIV acquisition among U.S. men who have sex with men⁸ and among

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Conflicts of Interest: For the remaining authors, no conflicts of interest were declared.

African men in adult male circumcision trials.^{9,10} Similarly, results from four observational studies conducted among women in Zimbabwe,^{11,12} South Africa,¹³ and Rwanda¹⁴ showed that HPV infection increased risk of HIV acquisition twofold. Collectively, these data have led to a new concept in which HPV and HIV infections may be bi-directional, each increasing the risk of the other.¹⁵⁻¹⁹ However, an inherent problem with observational studies is that HPV and HIV infection may be associated for reasons other than biological interaction, such as residual confounding by sexual behavior. A randomized controlled trial is needed to definitively assess whether HPV prevention with a highly efficacious and relatively simple intervention decreases HIV acquisition. In studies to date, HPV infection with either low-risk or high-risk types appears to confer risk for HIV, implying that a vaccine directed at multiple HPV types is needed to reduce this risk. As such, the 9-valent HPV vaccine has recently completed international Phase III trials, demonstrating safety²⁰ and clinical efficacy.²¹

The purpose of the current Phase II Trial was to assess the feasibility of conducting a placebo-controlled randomized HPV vaccine trial in a female population at high risk for HIV and to estimate the prevalence and incidence of HIV, HPV, and other STIs by age.

Materials and Methods

Population

Women residing in the Western Cape, South Africa were enrolled from November 2012 to July 2013 in a preparedness study, the Efficacy of HPV Vaccine to Reduce HIV Infection (EVRI) Trial (NCT01489527). Participants were recruited from the Kraaifontein day hospital and the Bloekombos primary health care clinic by community workers and through word of mouth, flyers, and brochures. Study recruitment messaging invited women to participate in a vaccine study against cervical cancer. The informed consent form presented the link between HPV and HIV and informed potential participants that the study would be used to determine whether it would be possible to conduct a larger study in the future to evaluate the potential utility of the cervical cancer vaccine in preventing HIV infection. To encourage compliance with follow-up, women received compensation for time and transportation at each visit.

The enrolled population consisted of women who met the following eligibility criteria: a) ages 16-24; b) no abnormal Pap smear history; c) reported having vaginal intercourse; d) not currently pregnant or breastfeeding; e) HIV-negative; f) no autoimmune disease requiring steroid use; g) never had a splenectomy; h) not currently enrolled in an HIV prevention trial; i) no IV drug or crystal methamphetamine use in the past 6 months; j) no history of serious allergic reactions requiring medical attention; k) no allergies to aluminum, yeast, or benzonase; l) no previous HPV vaccination; m) willingness to comply with four scheduled visits within the next seven months; and n) agreed to use effective contraception during sexual intercourse for the vaccination period.

This study was conducted in accordance with ethics committee review and approved by the Institutional Review Boards of The University of South Florida and Stellenbosch University. South African policies and ethics approval regarding parental permission for children to take

part in research studies were followed. Parents provided consent for minor study participants (16-17 years old), including testing for HIV. Minors provided assent. Parents/legal guardians were informed of their child's HIV test results.

Study Protocol

A Phase II randomized controlled trial of Gardasil vs. placebo (saline), given per label, was conducted among females ages 16-24. After confirming trial eligibility and obtaining consent, the EVRI Trial consisted of: 1) completion of a study questionnaire and testing for pregnancy and HIV; and 2) randomization of HIV-negative, non-pregnant females to vaccine or placebo with active follow-up for seven months (one month post-dose three of the vaccine). Women with a positive pregnancy test or HIV test at enrollment were referred for clinical care and management.

Trial-eligible women were randomized 1:1 to receive Gardasil or placebo vaccines. All staff and study investigators were blinded to participants' vaccine status except the pharmacist dispensing the vaccine (SK). Vaccine was administered at enrollment, month 2, and month 6. Study participants were followed for one month after the last vaccine dose (through month 7). At month 7, individual unblinding occurred, and women randomized to the placebo group were offered Gardasil vaccine.

At each follow-up visit after randomization, urine pregnancy tests and rapid HIV tests were performed. Women with positive pregnancy tests were referred to care and removed from the study, as pregnancy is contraindicated for HPV vaccination. Women with a positive rapid HIV test were retested with two different confirmatory tests. Participants with a confirmed positive HIV test after the enrollment visit were referred to care and remained on trial.²²

At enrollment, sexual history, health, and sociodemographic characteristics were assessed by a tablet-based questionnaire using a computer-assisted self-interview available in English, Xhosa, and Afrikaans. In the enrollment questionnaire, 116 participants reported not having had vaginal intercourse, despite having reported previous vaginal intercourse during the eligibility screening. In-depth qualitative interviews with participants revealed confusion with the term "vaginal." Consequently, different types of intercourse were explained to participants to prevent future misreporting of sexual history. We then compared the prevalence of HPV, chlamydia, gonorrhea, syphilis, and HSV-2 between the group reporting a valid sexual history (n=351) and the "virgin group" (n=116) and found no significant differences.

HSV-2 antibody and syphilis testing was measured in serum at enrollment. At the enrollment and month 7 study visits, an additional blood specimen was collected to measure HPV antibodies. If a participant HIV seroconverted, CD4 counts were quantified from the specimen obtained at the visit when HIV was first detected. Gonorrhea and chlamydia urine testing was performed at enrollment and month 6. The external genitalia were examined for skin pigmentation, lesions, skin irritation, discharge, nodules, and condylomata at enrollment and month 7. A speculum exam of the vagina was conducted and, after collection of specimens, a digital vaginal exam was performed. Samples obtained from the vulva/labia

and endocervical/ectocervical specimens for HPV detection were obtained at enrollment and month 7 visit using a pre-wetted Dacron cotton swab placed in an STM collection vial (Digene Hybrid Capture test kit). Specimens for HPV analysis were archived at 4°C prior to testing. Cervical cytology specimens were obtained at enrollment using the SurePath method. If Pap smear results were greater than atypical squamous cells of undetermined significance (ASCUS)/low-grade squamous intraepithelial lesions (LSIL), the participant was referred to the local clinic for a repeat Pap smear and further clinical management as indicated. If Pap smear results were high-grade squamous intraepithelial lesions (HSIL), the participant was referred to the study gynecologist (HB) for colposcopy and clinical management as indicated.

Participants testing positive for HIV, syphilis, gonorrhea, or chlamydia were immediately contacted and provided with STI results and treatment as indicated. Counseling was offered to all study participants at each clinical visit, regardless of age, per South African guidelines, including provision of condoms at no cost.

Laboratory Analyses

HIV status was assessed by rapid testing using the Determine® HIV-1/2 Ag/Ab Combo immunoassay (Alere Healthcare Pty Ltd). HIV-positive specimens underwent confirmatory testing utilizing the Abbott AxSYM HIV Ag/Ab Combo (HIV Combo) and the BioMerieux VIDAS HIV DUO assays. CD4 and CD8 counts were assessed among HIV seroconverters through EDTA whole blood stained with BD Multitest CD3/8/45/4 reagent in BD Trucount bead tubes (BD Biosciences) and were analyzed on a BD FACS Calibur according to standardized procedures. For HPV analyses, DNA was extracted from 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). HPV genotyping was conducted on all specimens, regardless of PCR results, using the Linear Array HPV Genotyping Test (Roche Diagnostics), which detects 37 HPV genotypes (high-risk: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68; low-risk: 6, 11, 26, 40, 42, 53-55, 61, 62, 64, 66, 67, 69-73, 81, 82, 83, 84, 89, IS39).²³ The Linear Array method cannot detect HPV 52 co-infections in the presence of HPV 33, 35, or 58, so some HPV 52 co-infections may have gone undetected. Among the 402 women with cervical cell specimens, 5 had inadequate amount of sample for HPV detection, 6 were HPV β -globin negative and 391 were HPV β -globin positive.

Gonorrhea and chlamydia were detected in urine specimens using the Anyplex CT/NG real-time detection method (Seegene). Syphilis was detected with the Captia Syphilis (*T. pallidum*)-G assay (Trinity Biotech), and specimens were reported positive only if confirmed positive by a repeat test with the same assay.²⁴ HSV-2 antibody status was assessed using the Captia HSV-2 type-specific IgG enzyme-linked immunoassay (EIA; Trinity Biotech).

Protocol Monitoring and Safety Evaluation

Protocol monitoring was conducted by Moffitt Clinical Research Network (MCRN) staff. Monitoring included review of data entered into OnCore, tracking, regulatory documents,

and case reviews, including the informed consent process, source documentation, protocol deviations, and adverse event reporting. No serious adverse events were reported in this trial.

Statistical Analysis

The total planned clinical trial sample size was 400, with 200 participants randomized to each of the vaccine and placebo groups. We accounted for 5% attrition over the follow-up period to obtain a planned final sample size of 380 women completing 7 months on study post-randomization. Demographic and sexual behavior characteristics from the enrollment questionnaire were described for all enrollees. The non-randomized and randomized groups were compared with respect to demographic and behavioral characteristics using exact Pearson Chi-squared tests for categorical variables and Mann-Whitney tests to compare medians of continuous variables, with p-values computed using Monte Carlo methods. Relationships between prevalence of HPV and STIs and age were assessed using exact Cochran-Armitage Trend tests with Monte Carlo Estimation of two-sided p-values.

Results

Overall, 924 women were approached for study participation: 404 women were ineligible, 35 were unwilling to participate, and 6 women dropped from the study prior to enrollment (Figure 1). Ineligibility was predominantly due to being outside the eligible age range (34%), currently pregnant or breast feeding (23%), or self-reported HIV-positivity (19%) (Supplementary Table 1). Among the 479 enrolled women, a total of 77 were ineligible for the vaccine trial component of the study due to testing HIV-positive (n=57), pregnant (n=12), or both (n=1) at enrollment, and 7 dropped from the study prior to randomization. Of these 77 participants, 12 had incomplete surveys and were removed from analyses, resulting in 402 women randomized to receive vaccine or placebo and 65 not randomized (Figure 1). Parental consent and participant assent was obtained from the 37 enrolled females under age 18, 29 of whom were eligible for randomization. Among randomized participants, 91% completed the three-dose vaccination series, with pregnancy being the predominant reason for trial discontinuation.

The median age of enrolled participants was 20 years (range 16-24). The majority of participants were single (95%), black (94%), and had not passed grade 12 (61.4%) (Table 1). Median age of first vaginal sex was 17, median number of lifetime male sexual partners was three (range 1-57), 25% reported current depo medroxyprogesterone acetate (Depo-Provera) use, and 50% reported having ever been pregnant (Table 1). No significant differences between the non-randomized and randomized groups were observed with respect to demographic and sexual health characteristics.

Prevalence was high for syphilis (6.2%), gonorrhea (10.9%), and chlamydia (32.8%), and for HSV-2 antibodies (46.5%) (Table 2). Only 19% of participants were negative for both HPV and a treatable STI (gonorrhea, chlamydia, syphilis). Forty percent of participants were HPV-positive with no co-infection with a treatable STI, and 31.2% were co-infected with HPV and a treatable STI. Normal cervical cytology was observed among 76.4% of participants, with a majority of the abnormal cytology findings being low-grade lesions

(11.4% ASCUS, 11.7% LSIL) (Table 2). Prevalence of 1 HPV types was 71%, with 50% harboring 2 concurrent HPV types (Table 3). The most commonly occurring types were high-risk HPV types 16 (14%), 52 (11%), and 58 (10%). Twenty percent of participants tested positive for one of the genotypes targeted by the 4-valent HPV vaccine (6/11/16/18), 2% for two types, and no study participant was positive for three or four types at enrollment. Twenty-eight percent of participants were positive for one of the genotypes targeted by the 9-valent HPV vaccine (6/11/16/18/31/33/45/52/58), 11% for two types, 2% for three types, 1% for four types, and none were positive for 5 HPV vaccine-related types at enrollment. Three percent of participants had infection with only HPV types found in the 4-valent HPV vaccine (HPV 6/11/16/18) while 9% had infection with only HPV types found in the 9-valent vaccine (HPV 6/11/16/18/31/33/45/52/58).

Figure 2a presents age-stratified prevalence of HPV by group (any, high-risk, low-risk, 1 4-valent vaccine type, 1 9-valent vaccine type, and multiple concurrent infections with any type). Regardless of HPV grouping, prevalence was highest among the youngest women (83% among ages 16-17), decreased with age, and was lowest among women age 24 years (60%). Age-related decreases in HPV prevalence were statistically significant for, 4-valent HPV vaccine type ($p < 0.05$). Similarly, prevalence of other STIs (Figure 2b) was highest among younger women and significantly decreased with age for gonorrhea and chlamydia infections. Although differences were not statistically significant, the prevalence of syphilis was highest at age 16 (20%) and was ~2-3-fold lower among women 17 years (3-10%). Similarly, gonorrhea infection peaked at ages 18-19 (20%), and chlamydia infection peaked at age 18 (43%) and remained high, with a significant decline observed at ages 23-24 (19%). As expected, HSV-2 antibody prevalence significantly increased with age.

Discussion

We successfully established the infrastructure and procedures to conduct a placebo-controlled randomized HPV vaccination trial with high rates of population acceptance. Results from this study demonstrate the feasibility of conducting future HPV vaccine trials to reduce HIV acquisition in high-risk populations and highlight the need to intervene at young ages and concurrently screen and treat multiple STIs. Interventions focused on only one susceptibility factor may result in only marginal declines in HIV incidence.^{3,25}

HPV vaccines are designed to prevent initial infection and are therefore recommended for a sexually naïve population, typically ages 11-12 years. The data presented here clearly demonstrate rapid cervical HPV acquisition after sexual debut and highlight the need for early intervention. In the current study, cervical HPV prevalence peaked in the youngest age groups (<18 years), consistent with the pattern observed among adolescents from other high-risk populations.^{26,27} While pre-sexual exposure vaccination is preferable in public health settings, trials designed to test efficacy of HPV vaccines to prevent HIV must be conducted among sexually active populations but prior to the steep rise in the HIV acquisition rate. Data presented here suggest that younger (<18 years), rather than older (>24 years) females need to comprise a future clinical trial population to achieve maximal HPV vaccine protection while maximizing trial efficiency. Fortunately, HPV infection can be prevented with a three-dose vaccination series, with efficacy approaching 100% for the 4-valent and 9-

valent vaccines. The 4-valent vaccine has also proven highly effective with a long duration of efficacy.^{21,28-33}

In addition to HPV, consideration must be given to other factors known to increase susceptibility to HIV. Lack of control of these factors may mask any potential efficacy of HPV vaccination. In the current trial, prevalence of multiple bacterial and viral STIs was high. The extremely high prevalence of bacterial STIs is not consistently observed across countries in sub-Saharan Africa but instead appears to be country- and region-specific.^{12,34-36} In settings such as South Africa, where STI prevalence is high in the general population, STI treatment needs to be a component of a multiple intervention strategy to reduce HIV.^{25,37}

HIV-positivity was self-reported among 18.6% (75/404) of study-ineligible women, with another 12% (57/479) of study-eligible women testing HIV-positive. We observed highest HPV and bacterial STI prevalence among those under age 18 years ($p < 0.05$, data not shown), prior to the rise in HIV acquisition seen in the general population, as would be expected if HPV and bacterial STIs indeed contribute to HIV risk. Fortunately, in the Western Cape, we experienced strong parental support for enrollment of their adolescent daughters to this trial.

Given the high prevalence of STIs with few lifetime male sexual partners, our data also suggest the need to include both genders in STI and HIV prevention efforts, as even one male sexual partner places a woman at substantial risk in settings where HIV and STIs are endemic. STI control and HPV vaccination, combined with regular HIV screening and referral to early treatment, is likely needed to halt the HIV epidemic in South Africa.³⁸

The EVRI Trial had a short duration with limited follow-up time; therefore, clinical efficacy in reducing HIV acquisition cannot be assessed. Our findings may not be generalizable to all South African women and likely do not represent STI and risk conditions of women in other sub-Saharan countries. We encountered comprehension issues in defining sexual activity that led to errors in questionnaire reporting. Therefore, we designed educational posters for participants mid-study, significantly improving the consistency of questionnaire responses. The prevalence of other genital tract infections, such as trichomonas, and other parasitic infections, or non-STI viral infections, was not measured. Finally, attrition from the study (9%) was primarily due to pregnancy, a factor that must be considered in powering future vaccine intervention trials to prevent HIV.

Despite having a small staff, we met accrual goals within a relatively short period of time (~8 months) and maintained trial protocol compliance through the consenting process, source documentation of clinical procedures, laboratory testing, and completion of the vaccine series (91%). Successful implementation of the study was due in large part to acceptance and interest of the trial in the community. A larger definitive trial demonstrating HPV vaccine efficacy in combination with STI control and HIV screening and treatment is needed. If shown efficacious, HPV vaccination could be added to the list of validated approaches to reduce the risk of HIV in addition to 'treatment as prevention' interventions, adult male medical circumcision,³⁹⁻⁴¹ condom use,⁴² antiretroviral microbicide,⁴³ and oral

PrEP use.^{44,45} (See Supplementary Digital Content, Appendix 1 for information on the ethical review.)

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors acknowledge the contributions of Charlotte Lawn, Wendy Adendorff, Zukiswa Gloria Ncume, Kayoko Kennedy, Dale Barrios, Jeannie Vaughn, David Jackson, Shahieda Isaacs, Nafisah Chotum, Donna J. Ingles, and all study participants, without whom this study would not have been possible.

Source of Funding: Merck (IISP39582) was the main sponsor of this trial and provided the study product. This work was also supported by the National Cancer Institute at the National Institutes of Health (Cancer Prevention Fellowship R25T CA147832 to S.L.S.). A.R.G. is currently receiving a grant (IISP39582) from Merck and is on the Speaker's Bureau of Merck.

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Figure 1.
Profile of the randomized, placebo-controlled Phase II EVRI Trial.

Figure 2. Prevalence of HPV and STIs by Age

a) Prevalence of HPV infection by age (p-values based on test of trend; * denotes significance at the 0.05 level).

b) Prevalence of STIs (syphilis, gonorrhea, chlamydia, HSV-2) by age (p-values based on test of trend; * denotes significance at the 0.05 level).

Table 1
Demographic and sexual health characteristics of women in the EYRI Trial

	Enrolled n=467	Non-randomized ^a	Randomized n=402	P-value ^b
Median age (range)	20 (16-24)	20 (16-24)	20 (16-24)	0.60
Survey language				0.34
	English	64 (98.5)	386 (96.0)	
	Xhosa	1 (1.5)	4 (1.0)	
	Afrikaans	0 (0.0)	12 (3.0)	
Race				0.68
	Black	63 (96.9)	375 (93.3)	
	Mixed	2 (3.1)	22 (5.5)	
	White	0 (0.0)	3 (0.7)	
	Other	0 (0.0)	2 (0.5)	
Marital status				1.00
	Single	62 (95.4)	383 (95.3)	
	Married	1 (1.5)	7 (1.7)	
	Living Together	2 (3.1)	10 (2.5)	
	Widowed	0 (0.0)	2 (0.5)	
Education				0.27
	Grade 7	4 (6.2)	20 (5.0)	
	Grade 8-12	42 (66.2)	220 (54.7)	
	Passed Grade 12	8 (12.3)	80 (19.9)	
	Some college/tech	10 (15.4)	82 (20.4)	
Median age of first vaginal sex ^c (range)	17 (1-21)	16 (14-20)	17 (1-21)	0.68
Current birth control use				0.18
	Oral Contraceptives	25 (38.5)	193 (48.0)	
	IUD/Loop/Coil	3 (4.6)	14 (3.5)	0.71
	Depo Provera	1 (1.5)	2 (0.5)	0.36
	Condoms	11 (16.9)	105 (26.1)	0.12
Ever been pregnant	107 (22.9)	11 (16.9)	96 (23.9)	0.27
	233 (49.9)	34 (52.3)	199 (49.5)	0.69

	Enrolled	Non-randomized ^a	Randomized	P-value ^b
Median no. of pregnancies (range)	n=467 1 (1-16)	n=65 1 (1-3)	n=402 1 (1-16)	0.39
Median lifetime no. of male sexual partners ^{c,d} (range)	3 (1-57)	3 (1-21)	3 (1-57)	0.06
Had vaginal sex in the last 6 months ^c	270 (76.9)	38 (88.4)	232 (75.3)	0.08
Condom use during vaginal sex in the last 6 months ^c				
Always	120 (34.2)	21 (48.8)	99 (32.1)	0.14
More than half the time	34 (9.7)	2 (4.7)	32 (10.4)	
Half the time	42 (12.0)	7 (16.3)	35 (11.4)	
Less than half the time	25 (7.1)	3 (7.0)	22 (7.1)	
Never	49 (14.0)	5 (11.6)	44 (14.3)	
No Sex	81 (23.1)	5 (11.6)	76 (24.7)	
Ever received presents/money/drugs for sex	14 (3.0)	1 (1.5)	13 (3.2)	0.70

^a Non-randomized: HIV-positive, pregnant, or dropped out prior to randomization (n=77); reduced by n=12 due to incomplete surveys;

^b P-values for differences between the non-randomized and randomized groups;

^c Sample size reduced due to errors in reporting sexual behavior (n=116): Enrolled (n=351), Non-randomized (n=43), Randomized (n=308).

^d Sample size further reduced by response of zero lifetime number of sexual partners (n=4): Enrolled (n=347), Non-randomized (n=42), Randomized (n=305)

Table 2
Sexually transmitted infection and abnormal cervical cytology prevalence among
randomized participants (n=402) at enrollment

	n	%
Cervical cytology		
Normal	307	76.4
ASCUS	46	11.4
ASCUS-HPV+	1	0.3
LSIL	47	11.7
HSIL	1	0.3
Syphilis		
Negative	376	93.5
Positive for only syphilis	6	1.5
Positive for Syphilis + other STI	19	4.7
Missing	1	0.2
HSV-2		
Negative	214	53.2
Positive for only HSV-2	98	24.4
Positive for HSV-2 + other STI	89	22.1
Missing	1	0.2
Gonorrhea		
Negative	357	88.8
Positive for only gonorrhea	5	1.2
Positive for gonorrhea + other STI	39	9.7
Missing	1	0.2
Chlamydia		
Negative	269	66.9
Positive for only chlamydia	50	12.4
Positive for chlamydia + other STI	82	20.4
Missing	1	0.2

Note: ASCUS: atypical squamous cells of undetermined significance, LSIL: low-grade squamous intraepithelial lesions, HSIL: high-grade squamous intraepithelial lesions

Table 3
HPV prevalence among women in the randomized group at enrollment (n=391)

	n	%
Any HPV type	279	71.4
Any high-risk type	219	56.0
16	55	14.1
18	25	6.4
31	19	4.9
33	7	1.8
35	35	9.0
39	17	4.4
45	24	6.1
51	34	8.7
52	44	11.3
56	13	3.3
58	39	10.0
59	28	7.2
68	25	6.4
Any low-risk type	200	50.2
6	12	3.1
11	4	1.0
26	11	2.8
40	6	1.5
42	7	1.8
53	23	5.9
54	22	5.6
55	16	4.1
61	33	8.4
62	29	7.4
64	1	0.3
66	28	7.2
67	8	2.1
69	4	1.0
70	17	4.4
71	5	1.3
72	5	1.3
73	16	4.1
81	28	7.2
82	10	2.6
83	18	4.6
84	25	6.4
89	24	6.1

	n	%
IS39	7	1.8
Coinfection with multiple types	196	50.1
4-valent vaccine types (6/11/16/18)		
Positive for 1 type	78	20.0
Positive for 2 types	9	2.3
9-valent vaccine types (6/11/16/18/31/33/45/52/58)		
Positive for 1 type	108	27.6
Positive for 2 types	41	10.5
Positive for 3 types	9	2.3
Positive for 4 types	4	0.8