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Prevalent Herpes Simplex Virus-2 Increases the Risk of Incident Bacterial Vaginosis in Women from South Africa

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Abstract

Studies have shown that women diagnosed with herpes simplex virus-2 (HSV-2) have a higher risk for bacterial vaginosis (BV) infection. We investigated the presence of HSV-2 infections as a risk factor for incident BV infections in high risk, Human immunodeficiency virus (HIV) uninfected women enrolled in a HIV prevention trial in Durban, South Africa. The Vaginal and Oral Interventions to Control the Epidemic trial was a multicentre, double blinded, randomized controlled trial which was designed to estimate the effectiveness of daily treatment with vaginal tenofovir gel, oral tenofovir disoproxil fumarate and oral Truvada in preventing HIV-1 infection in women. Women provided samples for the diagnosis of HSV-2 and BV. The presence of HSV-2 antibodies was detected using HerpeSelect™ ELISA IgG. Bacterial vaginosis was diagnosed using the Nugent scoring system. To assess the risk of BV incidence, modelled as a time-dependent variable, we used the Andersen-Gill model with robust variance estimation and Efron methods for ties. Overall, 2750 women were enrolled in the VOICE trial at our study sites. Women who had a HSV-2 infection at enrolment were shown to be at increased risk for incident BV infections (adjusted hazard ratio 1.17, 95% CI 1.08, 1.27, $p \leq 0.001$). In addition, being of a young age, being unmarried and having a partner that has other partners were significantly associated with subsequent BV infection. Our findings therefore advocate the need for strengthening STI prevention efforts among women in high burden STI settings.

Keywords

Herpes simplex virus type −2; Bacterial vaginosis; High-risk women; HIV infection

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Authors' Contribution NSA developed the concept with input from MN. NSA performed the data interpretations. GR was the clinical trials unit PI for the study. MN completed the statistical analysis. NSA wrote the paper with input from MN, SN and GR.

Compliance with Ethical Standards

Conflict of interest All authors mentioned in the manuscript declare that they have no conflicts of interest.

Informed Consent Written informed consent was obtained from all women that were enrolled in this study. The study was approved annually by the South African Medical Research Council Ethics Committee as well as the Medicines Control Council.

Introduction

Herpes simplex virus-2 (HSV-2) is one of the most prevalent viral sexually transmitted infections (STIs) globally, with rates as high as 78% in women [1, 2]. According to the World Health Organization (WHO), 536 million people aged 15–49 are infected with HSV-2, the causative agent of genital herpes [3]. In addition, approximately 23.6 million people in this age group become newly infected with HSV-2 each year [3]. Women in Sub-Saharan Africa have been shown to harbour the highest number of HSV-2 infections (i.e., 70%) [3]. In South Africa, the prevalence of HSV-2 infection is reported to be 40–70% [4, 5]. Bacterial vaginosis (BV) represents the main cause of abnormal vaginal discharge in women of reproductive age [6], and has been linked to considerable gynecologic and obstetric morbidity such as preterm delivery, pelvic inflammatory disease (PID) and upper genital tract infections [7]. The prevalence of BV has been reported to be as high as 51% among African women [8].

Many epidemiological studies have found a strong association between HSV-2 infection and Human Immunodeficiency Virus (HIV) infection [9]. In a more recent study by Abbai et al. [5], the prevalence of HSV-2 and HIV co-infection was reported to be 41% in South African women. Additionally, HSV-2 infection may also enhance host susceptibility to other STIs as well as lead to alterations in vaginal bacterial microbiota [10].

Bacterial vaginosis (BV) has also been found to be a significant risk factor for HIV-1 acquisition [7]. It has also been suggested that BV may be a sexually associated condition since many studies have shown that BV positive individuals share similar risk factors with that of STI infected individuals [11].

According to Nagot et al. [12] HSV-2 infected women have a higher prevalence of BV when compared to uninfected women. A study by Kaul et al. [13] showed that women with prevalent HSV-2 infection have a higher incidence of BV compared to HSV-2 uninfected women. However, the above-mentioned studies were conducted using a targeted population, a cohort of sex workers, therefore the data derived from these studies are not applicable to the general population. In this study, we will determine the association between prevalent HSV-2 infections with the risk of incident BV infections in a large cohort of HIV uninfected women that had enrolled to participate in a HIV prevention trial in Durban, South Africa.

Methods

Study Sites and Population

This study was performed as a secondary analysis of the recently completed Vaginal and Oral Interventions to Control the Epidemic (VOICE) trial (Clinicaltrials.gov # NCT00705679). The VOICE trial was a multicentre, double blinded, randomized controlled trial which was designed to estimate the effectiveness of daily treatment with vaginal tenofovir (TVF) gel, as compared with placebo gel, and of oral tenofovir disoproxil fumarate (TDF) and oral Truvada (TDF-FTC), as compared with oral placebo, in preventing sexually acquired HIV-1 infection in women and to assess the safety profiles of each of the active treatments. The products tested were not shown to protect against HIV-1 infection [14]. The

trial was conducted from September 2009 through August 2012, at 15 sites in South Africa, Uganda and Zimbabwe. This study is based on the data collected from the 7 South African Medical Research Council clinical research sites (CRSs) in Durban, South Africa.

Participants were recruited from a variety of sources such as primary healthcare clinics, and family planning clinics, as well as various community-based locations. Participants were also referred to the study by peer educators [15], health and social service providers serving the target study population. Briefly, the inclusion criteria were women aged 18–45 years who were HIV negative, neither pregnant nor breast-feeding and who reported recent vaginal intercourse, were using effective contraception, and had normal renal, hematologic, and hepatic function [14]. The study protocol and informed consent forms were approved by local Institutional Review Boards/Ethics Committees (IRBs/ECs) prior to use. Written informed consent was obtained from all study participants.

Study Procedures

A detailed description of the study procedures are provided elsewhere [14]. Briefly, during study interviews, information on demographics, medical and menstrual history and sexual behaviour was obtained from enrolled women. This information was collected by interviewer administered questionnaires and audio computer-assisted self-interview (ACASI). The data recorded was based on self-report. Clinical procedures were conducted at screening, enrolment and during study follow-up visits. Participants underwent both physical and pelvic examinations. Samples were collected for the diagnosis of HSV-2, syphilis, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, and BV infections. Ecto- and endocervical cells were also collected for Papanicolaou (Pap) smear analysis. Women were provided with HIV counselling and testing, ongoing HIV/STI risk reduction counselling, condoms, and treatment of STIs and reproductive tract infections (RTIs) such as BV and candida. Women were also advised to use a reliable method of contraception during the study and several contraception methods were available at the research site clinics. Participants were followed up monthly for a maximum of 36 months, with a minimum follow-up period of 12 months for those enrolled last.

Laboratory Procedures

Testing for HIV was conducted at every monthly visit, whereas testing for STIs and RTIs were conducted at baseline, annually, at study end and when clinically indicated. Two HIV rapid tests were conducted on whole blood samples collected by venipuncture by use of the Determine HIV-1/2 (Abbott Laboratories, Japan) and Unigold™ Recombigen® HIV. The presence of HSV-2 antibodies was detected using HerpeSelect™ ELISA IgG (Focus Technologies, Cypress, CA, USA) with a cut-off value of 3.5. Blood samples were used to diagnose syphilis using the rapid plasma reagin [RPR] and *Treponema pallidum* haemagglutinin [TPHA], Randox Laboratories, Crumlin, UK) tests. Urine specimens were collected for detection of *N. gonorrhoeae*, and *C. trachomatis* infections by strand displacement amplification using the BDProbeTec™ ET SDA Assay (Becton–Dickinson Microbiology Systems, USA). *T. vaginalis* testing was done using the Genzyme Rapid Trichomonas test with vaginal swabs.

Measures

Outcome—The outcome of interest in this analysis was incidence of BV measured using the Nugent scoring system [16]. Scores of 0–3 were considered as negative, 4–6 intermediate and 7–10 as positive BV status. The following quality control checks were in place for the Nugent scoring: in-house reproducibility checks (100%) were conducted by laboratory staff trained on reading BV slides. In addition, the slides and scores were assessed by an external laboratory which confirmed the scores read by the in-house laboratory. Women were tested for BV at the screening visit. If they tested BV positive they had a 56-day window to receive treatment and be BV symptom free before enrolment. After enrolment the gram stain test was repeated at semi-annual, annual and product use end visit (PUEV). Woman-years of observation for incidence rate calculations were determined from enrolment date to date of gram stain testing. Being infected with BV was a recurrent event and a special statistical technique—Anderson-Gill Models—was used to account for this.

Predictor—Testing for HSV-2 infection was conducted at the study end on enrollment and PUEV plasma archive specimens. We used HSV-2 at enrolment as a predictor variable for BV infection in women who were BV negative at enrolment. Herpes simplex virus type 2 was considered as negative for HerpeSelect EIA < 0.90, equivocal 0.90–1.89 and positive ≥ 1.1. This was later dichotomized as negative (< 1.1) and positive (≥ 1.1).

Confounders—Informed by the literature [12, 13, 17, 18] we controlled for the following factors at enrolment confounding the association between HSV-2 status and incidence of BV: age (< 25, 25–34, ≥ 35), marital status (married vs not married), vaginal sex in last 4 weeks (yes/no), coital frequency or number of sex acts in last 7 days, condom use in sex acts last 7 days (yes/no), type of condom used (male, female, none), circumcision of partner, partner having other partners and being diagnosed with a sexually transmitted infection (syphilis, *N. gonorrhoeae, C. trachomatis* and *T. vaginalis)*.

Statistical Analysis

Differences in categorical variables by HSV-2 status (Table 1) and BV status at screening visit (Table 2) were analysed using Chi squared tests. We used the Andersen-Gill (A-G) model with robust variance estimation and Efron methods for ties [19] to assess the risk of BV incidence, modelled as a time-dependent variable. The risk of BV acquisition was conditioned on HSV-2 status at enrolment and adjusted for socio-demographic variables (age, marital status, partner having other partners, circumcision of partner), behavioural factors (sexual activity in the last 7 days, frequency of sex acts in last 4 weeks, condom use at last sex), abnormal pap smears, and STIs (*N. gonorrhoeae*, *C. trachomatis*, *T. vaginalis*, syphilis). Follow-up time was right-censored for women who HIV sero-converted. The proportionality assumption was assessed using schoenfeld, scaled schoenfeld and martingale residuals. There was no evidence of violation of the proportionality assumption. All models were confirmed as good fit using Cox-Snell residuals test of goodness.

Additional analyses were conducted using the commonly adopted approach of modelling time to first event. Results from the time to first event and from A–G models were very comparable; it changed neither the interpretation nor direction of association between

baseline HSV-2 prevalence and subsequent incident BV infections among the study participants. We thus focus here primarily on results from the more robust A–G models.

At the Durban sites of VOICE, 2750 women were enrolled, of whom one woman was excluded for being BV positive at enrolment, one participant had missing information on HSV-2 status at enrolment and 4 had no follow-up visits post-enrolment giving us a final sample size of 2744 for this analysis. All analyses were conducted using STATA version 13.1 (College Station, TX: StataCorp LP).

Results

Characteristics of the Study Population by HSV-2 Status at Enrolment

Table 1 represents the baseline characteristics of the study population by HSV-2 status at enrolment. Overall, the prevalence rate for HSV-2 among the women at enrolment was 57.5% (n = 1577/2744). The prevalence of HSV-2 significantly increased with age; it was highest (77.6%) in the oldest age women (≥ 35 years old) ($p < 0.001$). By marital status, 58.2% of unmarried women compared to 47.3% of married women were HSV-2 positive at enrolment ($p = 0.004$). There were no significant differences in the prevalence of HSV-2 by coital activity in the last four weeks ($p = 0.635$), but HSV-2 status significantly varied ($p = 0.041$) by coital frequency in the last seven days with the prevalence being highest (61.5%) among those with four or more sex acts in the last 7 days as compared to those women with few sex acts ($p = 0.041$). Women who had used condoms in last 7 days had the highest (59.6%) diagnosis of HSV-2, however, there was no significant difference in type of condom used. Diagnosis of HSV-2 at enrolment further significantly varied by whether a woman's partner had other partners or partner was circumcised. Abnormal Pap smear findings, being diagnosed positive for *N. gonorrheae* or *T. vaginalis* infections were other factors significantly associated with HSV-2 positive status.

Prevalence and Associated Factors of BV at Screening Visit Prior to Enrolment

Table 2 represents the characteristics of the study population by BV status at screening visit prior to enrolment. Overall, the prevalence of BV positive women at screening was 40.7% (n = 1117/2744) and a further 16.3% were BV intermediate (n = 446/2744).

Within the group of women who were younger than 25 years of age, were unmarried and reported using condoms during their sex acts in the last 7 days, a larger percentage of women tested positive for BV. However, there was no significant association with prevalent BV infections, abnormal papsmear findings and prevalent STIs in the study population.

Factors Associated with Incidence of BV

Table 3 presents hazard ratios of the risk of incident BV infection over the study period by HSV-2 status of women at enrolment. Being HSV-2 positive at enrolment was significantly associated with incident BV infection in both unadjusted [hazard ratio (HR) 1.19, 95% confidence interval (CI) 1.10, 1.29, $p < 0.001$] and adjusted models (HR 1.17, 95% CI 1.08, 1.27, $p < 0.001$). Among the socio-demographic factors we controlled for in the adjusted analyses: being 35 years or older ($p = 0.08$), not married ($p < 0.001$), partner not circumcised

and coital frequency in the last 7 days were all associated with increased likelihood of BV incidence. Whereas, being aged under 35 years ($p = 0.01$) and partner not having other partners ($p = 0.002$) was associated with significantly less likelihood of BV acquisition. In addition, syphilis ($p < 0.001$), *C. trachomatis* ($p = 0.01$) and *T. vaginalis* ($p = 0.05$) at enrolment were among the biological factors significantly associated with incident BV infection among women who were HSV-2 infected at enrolment.

Table 3 further shows that using the approach of time to first event (ignoring all subsequent incidents of BV), both the unadjusted (HR: 1.35, 95% CI: 1.21, 1.50, $p < 0.001$) and adjusted hazard ratios (HR 1.37, 95% CI 1.23, 1.53, $p < 0.001$) of the association between HSV-2 positivity at enrolment and subsequent incidence of BV infection were comparable albeit higher than in the A-G models.

Discussion

In this study, the prevalence of HSV-2 at enrolment was estimated at 57%. Our findings are similar to those published by Kenyon et al. [4] who reported a prevalence of 53.3% for HSV-2 in a cohort of young South African women. The possible biological mechanisms for the associated risk of BV infections in women that have prevalent HSV-2 infections is described by Cherpes et al. [17] It has been suggested that the presence of a genital herpes infection changes the physiological and immunological environment of the vagina. It has been hypothesized that the change in the vaginal environment facilitates the overgrowth of anaerobic bacteria which in turn decreases the survival of hydrogen peroxide producing lactobacilli. The decrease in vaginal lactobacilli increases the risk of BV [17].

Our study identified a significant association between prevalent HSV-2 infections and the acquisition of BV in line with other literature [12, 13, 17]. The smaller effect size we see in our analysis relative to what others found could have been influenced by requirements of the VOICE protocol which indicated that all women who screened BV positive had to be treated and be BV free within a 56-day window before enrolment. This excluded any individual who could have had a prevalent BV and HSV-2 infection at enrolment.

The limitations of our study are as follows. Firstly, our analysis was performed only with data from HIV negative women. It has been previously reported that immunodeficiency caused by HIV-1 infection increases the occurrence and severity of HSV-2 reactivations, thereby resulting in increased BV episodes in HIV-1-positive women [18]. For future studies, HIV-1 status will be an important consideration for describing the association between HSV-2 and future BV infections. As per the study protocol, diagnosis of HSV-2 was only conducted at enrolment and at the end of study, therefore we were limited by the data to account for women who became HSV-2 infected during the trial. In addition, we are aware that confirming the results of the ELISA for HSV-2 with a Western blot would have provided a more accurate account on the actual number of HSV-2 infections, however, this was not a protocol requirement for the VOICE trial. However, in the VOICE trial HSV-2 diagnosis was made using the second- generation HerpesSelect ELISA from Focus Diagnostics. Focus Diagnostics had released the second-generation HerpeSelect ELISA based on the poor performance of the first-generation HerpesSelect EIA. Mark et al. [20],

had found that samples that had discordant EIA and Western blot HSV-2 results using the first generation test, were no longer discordant when using the second-generation kit. Thus, it appears that the second-generation Focus ELISA is more specific than the first-generation test.

In this analysis we had data that could have multiple failure events per subject, for which statistical methods that are able to account for possible correlation within subjects and discontinuous risk intervals like the A–G models are appropriate [19, 21]. Others adopt more naïve approaches such as modeling time to first event or modelling the risk intervals as continous [22]. As a sensitivity analysis we did run time to first event models and found results thereof to be very comparable to what we got from the A–G models (Table 3). Hence our primary focus was on results from the A–G models as A–G models have been found to be reliable and efficient in modelling recurrent events [23, 24]. Our findings however are limited to the extent to which the recurrent events may or may not have been independent, which is a main assumption of the A–G models. Another limitation of this analysis relates to the timing of the BV assessments that were done at the screeining then at the semi-annual, annual and product use end visits. It is thus likely for some BV episodes to have been missed given the long intervals between BV assessments.

The strength of our study is that the data used for our analysis was collected from women who reside in a highly prevalent HSV-2 and BV setting. Our findings therefore advocate the need for strengthening STI prevention efforts among women in high burden STI settings.

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Table 1

Characteristics at enrolment of VOICE study women by HSV-2 status, Durban, South Africa

	Total		HSV-2 negative		HSV-2 positive		p value
	N	%	n	%	n	%	
N	2744	100	1167	42.5	1577	57.5	
Age group							< 0.001
< 25	1523	55.5	791	51.9	732	48.1	
25–34	1047	38.2	337	32.2	710	67.8	
≥ 35	174	6.3	39	22.4	135	77.6	
Marital status							0.004
Married	182	6.6	96	52.7	86	47.3	
Not married	2562	93.4	1071	41.8	1491	58.2	
Had sex in last 4 weeks							0.635
Yes	2496	91.0	1058	42.4	1438	57.6	
No	248	9.0	109	44.0	139	56.0	
Number of sex acts last 7 days							0.041
0	825	30.1	386	46.8	439	53.2	
1	450	16.4	187	41.6	263	58.4	
2	589	21.5	242	41.1	347	58.9	
3	413	15.1	172	41.6	241	58.4	
≥ 4	467	17.0	180	38.5	287	61.5	
Number of sex acts last 7 days with condom							0.052
0	242	8.8	103	42.6	139	57.4	
1	424	15.5	172	40.6	252	59.4	
2	533	19.4	222	41.7	311	58.3	
3	339	12.4	141	41.6	198	58.4	
≥ 4	381	13.9	143	37.5	238	62.5	
Don’t know	825	30.1	386	46.8	439	53.2	
Sex act in last 7 days with condom							0.010
No	242	8.8	103	42.6	139	57.4	
Yes	1677	61.1	678	40.4	999	59.6	

	Total		HSV-2 negative		HSV-2 positive		p value
	N	%	n	%	n	%	
Don't know	825	30.1	386	46.8	439	53.2	
Type condom used for sex act in last 7 days							0.182
Male condom	2201	80.2	930	42.3	1271	57.7	
Female condom	19	0.7	12	63.2	7	36.8	
Unknown	524	19.1	225	42.9	299	57.1	
Does partner have other partners							0.014
Yes	336	12.2	125	37.2	211	62.8	
No	727	26.5	337	46.4	390	53.6	
Don't know	1681	61.3	705	41.9	976	58.1	
Is partner circumcised							0.004
Yes	735	26.8	306	41.6	429	58.4	
No	1627	59.3	669	41.1	958	58.9	
Don't know	382	13.9	192	50.3	190	49.7	
Pap smear findings							< 0.001
Normal	2209	80.5	966	43.7	1243	56.3	
Abnormal	454	16.5	158	34.8	296	65.2	
Not tested	81	3.0	43	53.1	38	46.9	
Diagnosed with Syphilis							0.12
Non-Reactive	2710	98.8	1157	42.7	1553	57.3	
Reactive	34	1.2	10	29.4	24	70.6	
Diagnosed with *N. gonorrheae*							0.003
Negative	2648	96.5	1142	43.1	1506	56.9	
Positive	94	3.4	25	26.6	69	73.4	
Not tested	2	0.1	0	0.0	2	100.0	
Diagnosed with *C. trachomatis*							0.325
Negative	2329	84.9	993	42.6	1336	57.4	
Positive	412	15.0	174	42.2	238	57.8	
Not tested	3	0.1	0	0.0	3	100.0	
Diagnosed with *T. vaginalis*							0.001
Negative	2558	93.2	1109	43.4	1449	2558	

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	Total		HSV-2 negative		HSV-2 positive		p value
	N	%	n	%	n	%	
Positive	183	6.7	56	30.6	127	183	
Not tested	3	0.1	2	66.7	1	33.3	

Note Percentages for the total column are column-wise, whereas for the HSV-2 negative and –positive percentages are row-wise

Table 2

Characteristics of VOICE women by baseline BV status

	Total		BV-negative		BV-intermediate		BV-positive		p value
	N	%	n	%	n	%	n	%	
N	2744	100	1181	43.0	446	16.3	1117	40.7	
Age group									0.002
< 25	1523	55.5	625	52.9	232	52.0	666	59.6	
25–34	1047	38.2	477	40.4	192	43.0	378	33.8	
≥ 35	174	6.3	79	6.7	22	4.9	73	6.5	
Marital status									0.002
Married	182	6.6	100	8.5	28	6.3	54	4.8	
Not married	2562	93.4	1081	91.5	418	93.7	1063	95.2	
Had sex in last 4 weeks									0.134
Yes	2495	91.0	1076	91.2	415	93.0	1004	89.9	
No	248	9.0	104	8.8	31	7.0	113	10.1	
Number of sex acts last 7 days									0.063
0	825	30.1	353	29.9	129	28.9	343	30.7	
1	450	16.4	188	15.9	65	14.6	197	17.6	
2	589	21.5	246	20.8	89	20.0	254	22.7	
3	413	15.1	187	15.8	66	14.8	160	14.3	
≥ 4	467	17.0	207	17.5	97	21.7	163	14.6	
Number of sex acts last 7 days with condom									0.299
0	242	8.8	112	9.5	34	7.6	96	8.6	
1	424	15.5	171	14.5	62	13.9	191	17.1	
2	533	19.4	227	19.2	88	19.7	218	19.5	
3	339	12.4	149	12.6	56	12.6	134	12.0	
≥ 4	381	13.9	169	14.3	77	17.3	135	12.1	
Don’t know	825	30.1	353	29.9	129	28.9	343	30.7	
Sex act in last 7 days with condom									< 0.001
No	242	8.8	112	9.5	34	7.6	96	8.6	
Yes	1677	61.1	716	60.6	283	63.5	678	60.7	

	Total		BV-negative		BV-intermediate		BV-positive		p value
	N	%	n	%	n	%	n	%	
	825	30.1	353	29.9	129	28.9	343	30.7	
Type condom used for sex act in last 7 days									0.723
Male condom	1591	58.0	686	58.1	0	0.0	636	56.9	
Female condom	12	0.4	3	0.3	269	60.3	6	0.5	
Unknown	316	11.5	139	11.8	3	0.7	132	11.8	
Not applicable	825	30.1	353	29.9	45	10.1	343	30.7	
Does partner have other partners									0.111
Yes	336	12.2	124	10.5	57	12.8	155	13.9	
No	727	26.5	330	27.9	110	24.7	287	25.7	
Don’t know	1681	61.3	727	61.6	279	62.6	675	60.4	
Is partner circumcised									0.996
Yes	735	26.8	319	27.0	118	26.5	298	26.7	
No	1627	59.3	701	59.4	264	59.2	662	59.3	
Don’t know	382	13.9	161	13.6	64	14.3	157	14.1	
Pap smear findings									< 0.001
Normal	2209	80.5	982	83.1	359	80.5	868	77.7	
Abnormal	454	16.5	153	13.0	78	17.5	223	20.0	
Not tested	81	3.0	46	3.9	9	2.0	26	2.3	
Diagnosed with Syphilis									0.088
Non-reactive	2710	98.8	1170	99.1	443	99.3	1097	98.2	
Reactive	34	1.2	11	0.9	3	0.7	20	1.8	
Diagnosed with *N. gonorrheae*									0.002
Negative	2648	96.5	1152	97.5	435	97.5	1061	95.0	
Positive	94	3.4	28	2.4	10	2.2	56	5.0	
Not tested	2	0.1	1	0.1	1	0.2	0	0.0	
Diagnosed with *C. trachomatis*									< 0.001
Negative	2329	84.9	1032	87.4	390	87.4	907	81.2	
Positive	412	15.0	147	12.4	55	12.3	210	18.8	
Not tested	3	0.1	2	0.2	1	0.2	0	0.0	
Diagnosed with *Trichomonas vaginalis*									< 0.001

	Total		BV-negative		BV-intermediate		BV-positive		p value
	N	%	n	%	n	%	n	%	
Negative	2558	93.2	1134	96.0	393	88.1	1031	92.3	
Positive	183	6.7	46	3.9	52	11.7	85	7.6	
Not tested	3	0.1	1	0.1	1	0.1	1	0.1	

Table 3

Association of HSV-2 status at enrolment with incident BV infections

	Using A–G models				Using time to first event models			
	HR	**[95 % CI]**		**p value**	**HR**	**[95 % CI]**		**p value**
Unadjusted								
HSV-2 negative	1.00				1.00			
HSV-2 positive	1.19	1.10	1.29	< 0.001	1.35	1.21	1.50	< 0.001
Adjusted								
HSV-2 negative	1.00				1.00			
HSV-2 positive	1.17	1.08	1.27	< 0.001	1.37	1.23	1.53	< 0.001

Adjusted for the following factors at enrolment: age, marital status, partner circumcised, had sex in last 4 weeks, number of sex acts in the last 7 days, partner has other partners, partner is circumcised, and STIs (syphilis, *N. gonorrhoeae C. trachomatis* and *T. vaginalis)*