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Herpes simplex virus type 2 cross-sectional seroprevalence and the estimated rate of neonatal infections among a cohort of rural Malawian female adolescents

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Abstract

Objective—To assess herpes simplex virus type 2 (HSV-2) seroprevalence among rural Malawian adolescent women and estimate the number of neonatal herpes infections among infants of these adolescents.

Methods—A longitudinal cohort study of adolescents (14–16 years at entry) residing in rural Malawi was initiated in 2007 with annual observation. HSV-2 testing was introduced in 2010. In this study, we (1) determined, using cross-sectional analysis, risk factors for positive serostatus, (2) adjusted for non-response bias with imputation methods and (3) estimated the incidence of neonatal herpes infection using mathematical models.

Results—A total of 1195 female adolescents (age 17–20 years) were interviewed in 2010, with an observed HSV-2 seroprevalence of 15.2% among the 955 women tested. From a multivariate analysis, risk factors for HSV-2 seropositivity include older age ($p=0.037$), moving from the baseline village ($p=0.020$) and report of sexual activity with increasing number of partners ($p<0.021$). Adjusting for non-response bias, the estimated HSV-2 seroprevalence among the total female cohort (composed of all women interviewed in 2007) was 18.0% (95% CI 16.0% to 20.2%). HSV-2 seropositivity was estimated to be 25.6% (95% CI 19.6% to 32.5%) for women

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who refused to provide a blood sample. The estimated number of neonatal herpes infections among the total female cohort was 71.8 (95% CI 57.3 to 86.3) per 100 000 live births.

Conclusions—The risk of HSV-2 seroconversion is high during adolescence, when childbearing is beginning, among rural Malawian women. Research on interventions to reduce horizontal and vertical HSV-2 transmission during adolescence in resource-limited settings is needed.

INTRODUCTION

Herpes simplex virus type 2 (HSV-2) is a leading cause of genital ulcer disease worldwide and an important cause of neonatal morbidity and mortality.¹² Most neonatal herpes infections result from exposure to HSV-2 in the genital tract during delivery, while in utero and postnatal infections occur less frequently.³ The risk of vertical transmission is substantially greater among women who acquire a primary HSV-2 infection late in pregnancy as compared with women with reactivated infections (50% vs <1%), since there is not adequate time to develop maternal antibodies to suppress viral replication before labour.⁴⁵ Infants who contract neonatal herpes infections experience 60% mortality rates if untreated and even with early high-dose intravenous acyclovir therapy considerable disability may arise.⁴⁶

A number of HSV-2 seroprevalence studies have been conducted among commercial sex workers, antenatal service attendees and sexually transmitted disease clinic attendees in sub-Saharan Africa due to their perceived high risk of infection and ease of sampling.⁷⁸ Relatively few studies have assessed HSV-2 prevalence and risk factors in a general population of female adolescents,^{9–13} despite the known high risk of sexually transmitted infections (STIs) in many sub-Saharan African countries.¹⁴ A cross-sectional study conducted among adolescents aged 14–24 years in a South African mining community found the prevalence of HSV-2 seropositivity increased 10% to 20% each year after sexual debut.¹⁰ Data on neonatal herpes burden in sub-Saharan Africa are minimal and estimates need to be extrapolated from seroprevalence data.¹⁵ No known study or assessment of HSV-2 serostatus has been conducted among female adolescents in Malawi or estimated the burden of neonatal herpes in this population.

The purpose of the present study was to (1) assess the prevalence of HSV-2 positive serostatus, (2) adjust seroprevalence estimates for non-response bias and (3) estimate the number of maternal and neonatal herpes infections in order to provide a comprehensive assessment of HSV-2 among rural female Malawian adolescents and their infants. These data may help guide the development of recommendations for the timing and targeting of behavioural interventions as well as informing potential immunisation programmes as HSV-2 vaccines are developed.

METHODS

Survey methods

The Malawi Schooling and Adolescent Survey (MSAS) is a longitudinal cohort study of 2650 adolescents residing in Machinga and Balaka districts, which are two contiguous rural districts in the southern region of Malawi. The initial 2007 sample consisted of 1764 randomly selected students (875 women and 889 men) interviewed at 59 randomly selected primary schools in Machinga and Balaka districts, who self-reported being between ages 14 and 16 in January 2007. The probability of a particular school being selected was proportional to its enrolment in 2006. At each school, approximately 30 students, stratified by gender and age, who attended standards 4–8 (the last 5 years of primary school) were enrolled in the study. An additional sample of 886 adolescents (463 girls and 423 boys) who were not enrolled in school during 2007 was drawn from the communities surrounding the

selected primary schools. These out-of-school respondents were identified through key informants located at the school or residents of the catchment villages. The sampling ratio of participants attending standards 4–8 relative to participants out of school was proportional to that observed in the 2004 Demographic and Health Survey (DHS) in Malawi.¹⁶ Follow-up interviews for the cohort were conducted annually. The study staff successfully reinterviewed 91%, 90% and 88% of the original sample in 2008, 2009 and 2010, respectively.

HIV and HSV-2 testing was introduced in 2010, the fourth round of data collection. Initially, dried blood spots (DBS) were considered for HSV-2 specimen collection and testing. However, a validation exercise implemented for a different study conducted by the principal investigators indicated that tests from DBS had a low sensitivity and specificity. Additional Institutional Review Board (IRB) clearance was necessary to change HSV-2 testing procedures for the adolescent cohort and approval was not obtained until after the start of the fourth round fieldwork. We attempted to recontact adolescents who had completed the survey but had not been offered blood collection for HSV-2 testing because they were interviewed prior to approval of the protocol amendment, but 49 respondents could not be relocated and therefore were not offered HSV-2 testing in 2010.

Laboratory methods

HIV status was assessed using a serial rapid testing algorithm to detect antibodies with Determine HIV 1/2 (Abbott, Japan) as the first test, Uni-Gold HIV 1/2 (Trinity Biotech, Ireland) as the second test and SD Bioline HIV 1/2 (Standard Diagnostics, Inc, South Korea) as the tiebreaking third test. Samples that were found reactive on the first two tests were considered HIV antibody positive. Samples that were non-reactive on the first test were considered HIV antibody negative. Any individual who was reactive on the first test but non-reactive on the second was retested and HIV status was determined with the third tie-breaker test. Sera were tested for antibodies to HSV-2 using the Kalon HSV-2 IgG2 ELISA (Kalon Biologicals, UK). Samples with optical density <0.9 were defined as HSV-2 negative, >1.1 were defined as HSV-2 positive and values 0.9–1.1 were defined as indeterminate as per manufacturer's instructions. Kalon ELISA-indeterminate samples were not retested, but represented only a fraction of cases (3.6%). A recent meta-analysis found the Kalon HSV-2 test for serum had a sensitivity of 95% (95% CI 93% to 97%) and specificity of 91% (95% CI 86% to 95%) in African samples.¹⁷ All study participants were offered pretest and post-test counselling. The results of the HSV-2 tests were given to participants at local health clinics with the appropriate counselling at the conclusion of data collection for that round.

Statistical methods

Risk factors for HSV-2 positive serostatus—The analyses presented in this study are limited to female adolescent respondents. Risk factors for HSV-2 seropositivity among women were examined using log-binomial regression models to obtain risk ratio estimates.¹⁸ Variables assessed in univariate and multivariate analyses included age, marital status, moving history, ethnic group, educational attainment, household wealth, pregnancy within last year, sexual activity and number of sexual partners in the last year, age at sexual initiation, consistent condom use and HIV status. We decided to not include HIV status in the final multivariate models due to overlapping risk factors and because we are not aware of studies suggesting that HIV infection increases risk of HSV-2 acquisition.¹⁹ All variables were assessed for collinearity, and missing data were retained in all analyses using the missing indicator method.²⁰ Since this is a cohort study, the fit of models using covariates from previous yearly surveys was also evaluated and no significant differences in model fit were found. We decided to use cross-sectional data from the 2010 interview, since we are

unable to determine the temporal sequence of covariate and HSV-2 seroconversion, since it is unknown when women sero-converted. p values were based on two-sided hypothesis tests and considered significant at $p = 0.05$.

Imputation techniques for individuals with unknown HSV-2 serostatus—

Imputation methods to correct HSV-2 seropositive prevalence estimates for non-response were used.^{21,22} We used all variables included in risk factor analyses to impute HSV-2 serostatus for women interviewed in 2010 but had unknown serostatus due to refusal, not being offered the test or having indeterminate results. In order to impute HSV-2 serostatus for women lost to follow-up prior to 2010, we used data from the baseline cohort survey (3 years prior), during which all participants were interviewed. Variables included in the imputation model for those lost to follow-up included age, marital status, ethnic group, migration, household wealth, educational attainment, report of sexual activity, ever pregnant, alcohol and condom use. Many of these socio-demographic and behavioural factors included in our models are also recommended by WHO and UNAIDS to adjust for testing refusals in HIV estimates.²³ Imputations were carried out with creation of five imputed data sets, a strategy recommended in studies without a large fraction of missing responses.²⁴ A prevalence estimate and its CIs for interviewed individuals (including tested, refusals, not offered and indeterminates) and for the total cohort (all women interviewed in the study in 2007, including interviewed individuals and those lost to follow-up) were obtained through a weighted average of imputed and observed prevalence estimates. All risk factor and multiple imputation analyses were performed using SAS V.9.1 (SAS Institute).

Modelling the incidence of maternal and neonatal HSV-2 infections—

The incidence of both maternal and neonatal HSV-2 infections was estimated using a simple catalytic model, which has been used elsewhere to estimate rates of congenital rubella syndrome and neonatal HSV-2 infections.^{15,25} A simple catalytic exponential decay model with a constant force of infection (per person incidence) per year, λ , was fitted to the HSV-2 seroprevalence data for tested women, interviewed women (including imputations) and the total female cohort (composed of all women interviewed in 2007) to calculate the force of infection separately for each group. The model is described by the following equation:

$$s(a) = \exp[-\lambda(a-15)],$$

where age, a , was restricted to be between 17 and 20 years, the age range of the sample in 2010. The proportion of the population seronegative at age a is described by $s(a)$ and this can be translated into the proportion seropositive, $p(a)$, through equation $1-s(a)$. This model was fit with maximum likelihood to estimate the force of infection, λ , and corresponding 95% CIs. This model requires the assumption that all women are not exposed to HSV-2 virus until the age of 15, and after this age, all women are exposed to HSV-2 with the same force of infection per year. Additionally, it is assumed that there is no reversibility of HSV-2 serostatus and women are immune to primary HSV-2 infection for life after seroconversion.

We can then estimate the risk of HSV-2 infection per pregnancy, P_m , using the force of infection, λ , as calculated above with the following equation:

$$P_m = s_m [1 - \exp(-\lambda(40/52))],$$

where s_m is defined as the average proportion of susceptible women between ages 15 and 24. This model assumes the force of infection among pregnant women is the same as for the general female population and the duration of pregnancy is 40 weeks.

Further, if we assume pregnant women can only vertically transmit HSV-2 if they are infected during the final 11 days of pregnancy based on the mean duration of viral shedding following a primary HSV-2 infection,¹⁵ we can also estimate the incidence of neonatal HSV-2 infection per live birth, P_n , using the following equation:

$$P_n = s_m [1 - \exp(-\lambda(11/365))]r,$$

where r is defined as the risk of transmission given a woman is infected during that last 11 days of pregnancy, which was assumed to be 50%.³¹⁵ This model assumes pregnancies among mothers infected with HSV-2 during the final 11 days of pregnancy are equally likely to result in a live birth as pregnancies of HSV-2 uninfected mothers.

Ethics statement

Written informed consent was obtained from all study participants before completing all interviews and additional informed consent was obtained for HSV-2 and HIV testing. For individuals who were enrolled in school in 2007, teachers were provided a letter to read to standards 4–8 and students were asked to inform their parents of participation in the study. Participants were asked to confirm their parents' approval as part of the informed consent process. For out-of-school participants, consent was first obtained from participants' parents or guardians, and then from the participants themselves. The study protocol was approved by the College of Medicine Research and ethics committee at the University of Malawi in Blantyre (reference: P.02/10/866) and the Population Council institutional review board in New York (protocol 481).

RESULTS

In 2007, 1338 female adolescents were enrolled in the MSAS cohort of whom 1195 were reinterviewed during the 2010 survey. A summary of the 2010 survey results is presented in table 1. A total of 955 (79.9%) interviewed women consented for HSV-2 testing of which 15.2% ($n=145$) had positive, 82.6% ($n=789$) negative and 2.2% ($n=21$) indeterminate HSV-2 results. The age-wise HSV-2 positive seroprevalence for tested women was 11.2% (95% CI 7.4% to 15.9%), 15.7% (95% CI 11.9% to 20.2%), 17.8% (95% CI 13.4% to 22.9%) and 18.6% (95% CI 11.9% to 27.0%) for 17, 18, 19 and 20-year-olds, respectively.

Table 2 presents univariate and multivariate risk factor analyses for HSV-2 positive serostatus. In the multivariate model, increasing age ($p=0.036$), moving from the baseline village ($p=0.020$) and report of sexual activity with increasing number of partners ($p=0.021$) were statistically significant independent risk factors for HSV-2 seropositivity.

Female adolescents who were interviewed in 2010, but refused to provide a blood sample for HSV-2 testing ($n=191$), were significantly more likely to not have completed primary school as compared with women who provided a blood sample for testing (63.2% vs 55.4%; $p=0.037$). Female adolescents who refused HSV-2 testing also had non-significant increases in other risk factors for HSV-2 positive serostatus, including report of sexual activity in the last year (67.9% vs 64.4%), being married (60.4% vs 54.2%), having moved from the baseline village (42.5% vs 38.3%) and being in the lowest wealth tertile (45.8% vs 39.5%).

Imputation methods were used to adjust the observed HSV-2 positive seroprevalence for non-response bias (table 3). The estimated HSV-2 positive seroprevalence among interviewed women with unknown status due to not being offered testing, refusing testing and indeterminate results was estimated to be 26.4% (95% CI 21.2% to 32.2%). The estimated seroprevalence among the refusals was 25.6% (95% CI 19.6% to 32.5%). A weighted average of the observed and imputation results estimated the prevalence of HSV-2 seropositivity to be 17.9% (95% CI 15.8% to 20.2%) among women interviewed in 2010 (n=1195). Imputation models also estimated the prevalence of HSV-2 positive serostatus to be 18.8% (95% CI 12.8% to 26.3%) among women lost to follow-up prior to HSV-2 testing in 2010. Combining imputation estimates and the observed results, the estimated HSV-2 seroprevalence among the total female adolescent cohort (n=1338; composed of women interviewed in 2007) in 2010 was 18.0% (95% CI 16.0% to 20.2%). The estimated age-wise HSV-2 seroprevalence for the total female cohort was 13.4% (95% CI 9.8% to 17.7%), 18.0% (95% CI 14.7% to 21.8%), 21.4% (95% CI 17.5% to 25.9%) and 18.5% (95% CI 12.8% to 25.6%) for 17, 18, 19 and 20-year-olds, respectively.

We also estimated the incidence of primary HSV-2 infection during pregnancy and neonatal infections. Figure 1 shows the fit of the simple catalytic exponential decay models. The force of infection or incidence of HSV-2 per 100 seronegative person-years was 4.99 (95% CI 4.27 to 5.71) for HSV-2 tested women, 5.80 (95% CI 4.77 to 6.82) for interviewed women and 5.82 (95% CI 4.64 to 6.99) among the total female cohort. Using these force of infection values, we estimate that 29.0 (95% CI 27.3 to 36.3), 33.6 (95% CI 29.6 to 42.0) and 33.7 (95% CI 28.7 to 42.9) primary HSV-2 infections per 1000 pregnancies occurred among tested women, interviewed women and the total cohort, respectively. Subsequently, we used the probability a female adolescent was infected during the final 11 days of pregnancy to estimate that 63.5 (95% CI 54.3 to 72.6), 71.7 (95% CI 59.0 to 84.3) and 71.8 (95% CI 57.3 to 86.3) primary neonatal HSV-2 infections per 100 000 live births occurred among tested women, interviewed women and the total female cohort, respectively.

DISCUSSION

In this study, we assessed HSV-2 seroprevalence and estimated the number of neonatal herpes infections for rural Malawi adolescent women and their infants. The prevalence of HSV-2 positive serostatus was 15.2% among female adolescents consenting for testing. Among tested women, the risk of being HSV-2 seropositive was independently associated with increasing age, moving from the baseline village and report of sexual activity with increasing number of sexual partners. These risk factors are well documented in the STI epidemiology literature.

Non-response bias in population-based surveys can occur because of testing refusal and population mobility leading to absenteeism.²⁶ There is a wide body of evidence that individuals absent for surveys, specifically DHSs, tend to have higher rates of risky sexual behaviours and higher risk of STIs.²⁷ However, there is limited and inconclusive evidence on how refusal to provide blood specimens for HSV-2 or HIV testing is related to risk, but studies suggest refusers have moderate increases in risky sexual behaviours.^{27,28} In this study, 16.7% of interviewed women refused to provide a blood sample and 10.7% women were lost to follow-up prior to 2010. Individuals who refused testing had significantly lower educational attainment and there were other non-significant increases in other risk factors, including report of sexual activity, being married, having moved from the baseline village and being in a lower wealth category. Imputation methods were used to account for these differences resulting in non-response bias for both the absentees and refusers. We estimated the prevalence of HSV-2 seropositivity was much greater among refusers (25.6%) and only slightly higher for absentees (18.8%) as compared with women who submitted for testing.

Despite markedly increased seroprevalence for refusers, these individuals only resulted in a slight increase in the seroprevalence estimate for the entire cohort composed of women interviewed in 2007 (18.0%) since a majority of women submitted for HSV-2 testing.

Using mathematical modelling techniques, we also estimated one case of neonatal herpes occurred per 1392 live births among the adolescent cohort in 2010. This estimate is double the infection rate of 1 per 3200 live births among the general US population and also the estimated rate used by a similar mathematical model among women 15–34 years in Kilifi, Kenya.¹⁵²⁹ This difference is likely due to the high force of infection during adolescence and the large proportion of women susceptible to HSV-2 infection during and soon after sexual initiation.

Most guidelines in developed-country settings recommend caesarean section for women developing a primary HSV-2 infection during the last 4–6 weeks of pregnancy; however, HSV-2 serological or PCR tests for diagnosis and safe and timely caesarean section are not currently available to most women in rural Malawi or other resource-limited settings.³⁰ As a result, intrapartum and neonatal intravenous acyclovir therapy, which is also indicated for mothers in developed-country settings when vaginal delivery is irreversible, is likely the safest approach to reduce the risk of vertical transmission for Malawian mothers with a suspected primary HSV-2 infection.⁵³¹ Additionally, primary HSV-2 infections could also be prevented by advising expectant mothers against unprotected genital–genital contact and genital–oral contact with sexual partners, especially during the final trimester.¹⁵

This study has several important limitations. First, we used cross-sectional data, which are unable to establish temporality of covariates and HSV-2 seroconversion. We would have also been unable to assess the temporal sequence of covariates using data from surveys conducted prior to testing in 2010, since it is not known when women seroconverted. Second, the results of this study are generalisable to adolescent women residing in two districts of rural southern Malawi and to adolescent populations with similar rates of HSV-2 acquisition, but we are hesitant to extrapolate our findings directly to a national Malawian sample or other populations in sub-Saharan Africa due to sparse data on HSV-2 seroprevalence. Additionally, the mathematical model estimating the number of neonatal herpes infections does not account for recurrent HSV-2 infections. As a result, we may have slightly underestimated the number of neonatal HSV-2 infections, but the risk of vertical transmission is estimated to be <1% for pregnant women with reactivated HSV-2 infections.⁵

Overall, the prevalence of HSV-2 seropositivity approaches one in five among rural Malawian adolescent women by 20 years of age. The high force of infection during adolescence, which is also the beginning of the childbearing years, is expected to produce the highest rate of neonatal herpes infection for rural Malawian women of any age group. The high prevalence of HSV-2 infection in this population is also a concern due to increased risk of HIV acquisition.¹⁸ Additional research is needed to evaluate the impact of HSV-2 prevention interventions among adolescents and their cost-effectiveness in rural Malawi.

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Key messages

- The risk of HSV-2 seroconversion is high during adolescence for rural Malawian women and approaches 20% by 20 years of age.
- Female adolescents who refused to provide a blood sample are estimated to have significantly greater HSV-2 seroprevalence compared with women submitting for testing.
- The risk of neonatal herpes infections is estimated to be high due to rapid seroconversion when childbearing is beginning for Malawian female adolescents.

Figure 1. Age-wise seroprevalence and maximum-likelihood estimates of herpes simplex virus type 2 force of infection with simple catalytic exponential decay model. Caption: b =force of infection. *Includes observed women and imputations for those not being offered testing, refusing testing and indeterminate results. **Includes interviewed women and imputations for interviewed women with unknown status and those lost to follow-up before 2010.

Table 1

Characteristics of interviewed female adolescents in 2010 (n=1195)

Characteristic	Frequency (%)
Age, years	
17	287 (24.0)
18	426 (35.7)
19	341 (28.5)
20	141 (11.8)
Marital status	
Ever married	660 (55.2)
Single	535 (44.8)
Migration history	
Residing in baseline village	725 (60.7)
Migrated from baseline village	470 (39.3)
Ethnic group	
Muslim	552 (46.2)
Christian	573 (48.0)
Other	70 (5.9)
Highest educational attainment	
No school/some primary	680 (56.9)
Completed primary	515 (43.1)
Alcohol use	
Yes	75 (6.3)
No	1115 (93.7)
Live birth within last year	
Yes	335 (28.0)
No	860 (72.0)
Sexual activity and no. of partners in 2010	
No sexual activity	420 (35.2)
1 Partner	723 (60.5)
2 Partners	52 (4.4)
Reported age at sexual initiation	
Under 14 years	253 (23.2)
15–17 years	709 (65.0)
18 years or older	128 (11.7)
Condom use (among sexually active)	
Reported at least some use	322 (41.5)
Reported no condom use	453 (58.5)
HIV serostatus	
Positive	31 (2.6)
Negative	1023 (85.6)
Unknown	141 (11.8)

Characteristic	Frequency (%)
HSV-2 serostatus	
Positive	145 (12.1)
Negative	789 (66.0)
Indeterminate	21 (1.8)
Unknown	240 (20.1)

HSV-2, herpes simplex virus type 2.

Table 2 Relative risk (RR) for HSV-2 positive seropositivity among tested[†] female adolescents (n=934)

Characteristic	Univariate RR (95% CI)	p Value	Multivariate RR (95% CI)	p Value
Age, years				
17 (ref)	1.0		1.0	
18	1.41 (0.90 to 2.19)		1.43 (0.92 to 2.23)	
19	1.59 (1.02 to 2.48)		1.61 (1.04 to 2.49)	
20	1.67 (0.98 to 2.83)	0.032*	1.58 (0.93 to 2.69)	0.036*
Marital status				
Single (ref)	1.0		1.0	
Ever married	1.55 (1.14 to 2.14)	0.006	1.07 (0.61 to 1.88)	0.801
Migration history				
Residing in baseline vlg. (ref)	1.0		1.0	
Migration from baseline vlg.	1.42 (1.04 to 1.95)	0.030	1.45 (1.06 to 1.99)	0.020
Ethnic group				
Muslim (ref)	1.0		1.0	
Christian	0.70 (0.51 to 0.95)	0.024	0.75 (0.55 to 1.03)	0.075
Other	0.63 (0.29 to 1.38)	0.248	0.59 (0.28 to 1.25)	0.170
Educational attainment				
None/some primary (ref)	1.0		1.0	
Completed primary	0.65 (0.48 to 0.90)	0.008	0.76 (0.53 to 1.07)	0.110
Wealth tertile				
Lowest tertile (ref)	1.0		1.0	
Middle tertile	0.98 (0.70 to 1.38)		1.04 (0.73 to 1.47)	
Highest tertile	0.85 (0.57 to 1.26)	0.440*	0.98 (0.65 to 1.48)	0.956*
Alcohol use				
No (ref)	1.0		1.0	
Yes	0.89 (0.46 to 1.73)	0.742	0.70 (0.37 to 1.32)	0.269
Live birth within last year				
No (ref)	1.0		1.0	
Yes	1.05 (0.75 to 1.45)	0.789	1.04 (0.73 to 1.47)	0.493
Sexual activity and no. of partners in 2010				
No sexual activity	1.0		1.0	
1 Partner	1.53 (1.07 to 2.18)		1.27 (0.66 to 2.45)	
2 Partners	2.79 (1.62–4.80)	<0.001	2.36 (1.12 to 4.96)	0.021*
Age at sexual initiation				
Under 14 years (ref)	1.0		1.0	
15–17 years	0.99 (0.70 to 1.42)		1.05 (0.74 to 1.49)	
18 years or older	0.69 (0.37 to 1.30)	0.023*	0.80 (0.42 to 1.53)	0.325*
Condom use				
None (ref)	1.0		1.0	
At least some	1.28 (0.94 to 1.76)	0.121	1.03 (0.73 to 1.45)	0.875

Characteristic	Univariate RR (95% CI)	p Value	Multivariate RR (95% CI)	p Value
HIV serostatus [‡]				
Negative (ref)	1.0	—	—	—
Positive	4.44 (3.20 to 6.17)	<0.001	—	—

HSV-2, herpes simplex virus type 2.

* p Value for test for trend.

[†] Individuals with indeterminate results were excluded.

[‡] HIV serostatus not included in final multivariate model.

Table 3

Observed and imputed HSV-2 seroprevalence for the female adolescent cohort

	Frequency (%)	% HSV-2 seropositive (95% CI)
Observed	934 (69.8)	15.5% (13.2 to 17.8)
Refusals, indeterminates and those not offered testing [*]	261 (19.5)	26.4% (21.2 to 32.2)
Lost to follow-up [*]	143 (10.7)	18.8% (12.8 to 26.3)
Total cohort (composed of all women interviewed in 2007) [†]	1338	18.0% (16.0 to 20.2)

HSV-2, herpes simplex virus type 2.

^{*} The results of imputation models.[†] Weighted average of observed and imputed prevalence estimates.