The epidemiology of HSV-2 infection and its association with HIV infection in four urban African populations

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Objectives: To estimate age- and sex-specific herpes simplex virus type-2 (HSV-2) prevalence in urban African adult populations and to identify factors associated with infection.

Design and methods: Cross-sectional, population-based samples of about 2000 adults interviewed in each of the following cities: Cotonou, Benin; Yaoundé, Cameroon; Kisumu, Kenya and Ndola, Zambia. Consenting study participants were tested for HIV, HSV-2 and other sexually transmitted infections.

Results: HSV-2 prevalence was over 50% among women and over 25% among men in Yaoundé, Kisumu and Ndola, with notably high rates of infection among young women in Kisumu and Ndola (39% and 23%, respectively, among women aged 15–19 years). The prevalence in Cotonou was lower (30% in women and 12% in men). Multivariate analysis showed that HSV-2 prevalence was significantly associated with older age, ever being married, and number of lifetime sexual partners, in almost all cities and both sexes. There was also a strong, consistent association with HIV infection. Among women, the adjusted odds ratios for the association between HSV-2 and HIV infections ranged from 4.0 [95% confidence interval (CI) = 2.0–8.0] in Kisumu to 5.5 (95% CI = 1.7–18) in Yaoundé, and those among men ranged from 4.6 (95% CI = 2.7–7.7) in Ndola to 7.9 (95% CI = 4.1–15) in Kisumu.

Conclusions: HSV-2 infection is highly prevalent in these populations, even at young ages, and is strongly associated with HIV at an individual level. At a population level, HSV-2 prevalence was highest in Kisumu and Ndola, the cities with the highest HIV rates, although rates were also high among women in Yaoundé, where there are high rates of partner change but relatively little HIV infection. The high prevalence of both infections among young people underlines the need for education and counselling among adolescents.

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Introduction

Sexually transmitted infections are known to facilitate HIV transmission through a number of direct, biological mechanisms [1]. In particular, genital ulcerative diseases increase HIV susceptibility through disruption of the mucosal barrier associated with ulceration, and increase HIV infectivity through enhanced genital shedding of HIV [2]. Herpes simplex virus type-2 (HSV-2) infection, a major cause of genital ulceration worldwide, has been found to be associated with HIV infection in several studies in Europe, the United States and Thailand [3–8]. Until recently, few studies of the epidemiology of HSV-2 infection in sub-Saharan Africa had been carried out, and these few reported high prevalence rates in general populations as well as high-risk populations [9–17]. HSV-2 infection may thus play an important role in the spread of HIV infection in sub-Saharan Africa.

A recent population-based cross-sectional survey in African cities [18] provided the opportunity to examine HSV-2 seroprevalence in four urban African populations with differing rates of HIV infection. The objectives of this substudy were to estimate prevalence of HSV-2 infection in each population by age and sex, to identify socio-demographic, behavioural and biological risk factors for HSV-2 infection, and to explore the association with HIV infection, adjusting for potential confounding factors.

Methods

Study population

Cross-sectional population surveys were carried out between June 1997 and March 1998 in two cities with high HIV prevalence (Kisumu, Kenya and Ndola, Zambia) and two with relatively low prevalence (Cotonou, Benin and Yaoundé, Cameroon). The sampling procedures, questionnaire, and sample collection and testing procedures were standardized across the four cities and are described elsewhere in this supplement [18]. Briefly, a cluster design was used to randomly select households within each city. The head of each selected household was interviewed, and individuals who had spent the previous night at the house were listed. Each household member aged 15–49 was interviewed regarding their socio-demographic characteristics and sexual behaviour. Study participants were requested to give a blood sample, which was tested serologically for HIV, syphilis and HSV-2, and a urine sample, which was tested for gonorrhoea and chlamydial infection. Women were also asked to insert a swab into the vagina, which was immediately innoculated into a culture medium for *Trichomonas vaginalis*. In addition, sera from a random sample of 100 men and 100 women in each city were tested for HSV-1 antibodies.

Laboratory methods

Serum samples were tested for HSV-1 and HSV-2 at the Institute of Tropical Medicine, Antwerp, using an HSV Type Specific IgG EIA (Gull Laboratories, Bad Homburg, Germany). According to the manufacturer, the sensitivity of this test was 86–98% and the specificity 97–100%, depending on the gold standard that was used. As the Gull test had not been previously validated for African sera, we re-tested a random sample of 256 samples from Cotonou, Yaoundé and Kisumu against a monoclonal antibody (mAb)-blocking enzyme-linked immunosorbent assay (ELISA), modified from a validated mAb-blocking radioimmunoassay [19]. Compared with the mab-blocking ELISA, the Gull test had a sensitivity of 93% and a specificity of 96%. Details of tests for HIV and other sexually transmitted diseases (STDs) are described elsewhere [18].

Statistical analysis

All data were double-entered and validated in EPI-INFO (CDC, Atlanta, Georgia, USA), and analysed in Stata 6 [20]. Analyses of HSV-2 infection were stratified by city and by gender, as the effect of various exposures on risk of HSV-2 infection may be modified by both these factors. Logistic regression was used to calculate age-adjusted odds ratios and 95% confidence intervals (CIs) for the association of HSV-2 with socio-demographic and sexual behaviour risk factors. The socio-demographic factors were marital status, circumcision status (men only; by clinical examination where this was performed, otherwise by self-report), education, religion, and occupation. The sexual behaviour factors were age at first sexual intercourse, lifetime number of partners, number of non-spousal partners in the past 12 months, and reported exchange of money for sex in the past 12 months. All subjects were included in analyses, whether or not they reported ever having had sexual intercourse. Variables significant at the 95% significance level in the age-adjusted analysis for any city were included in multivariate analyses, which again were stratified by city and gender. Statistical significance of odds ratios was assessed using the likelihood ratio test, and variables were retained in the model if they were statistically significant in any city. The association of HSV-2 infection with other sexually transmitted infections (HIV, active syphilis, gonorrhoea, chlamydia and *T. vaginalis* (women only), and reported history of genital pain or sores in the past 12 months (men only) were analyzed by including these variables in the final multivariate model.

Ethical approval

Ethical approval for the study was obtained from the national ethical committee in each of the four countries where the study took place, as well as from the ethical committees of the Institute of Tropical Medicine, Antwerp, the London School of Hygiene & Tropical Medicine, and the Population Council.

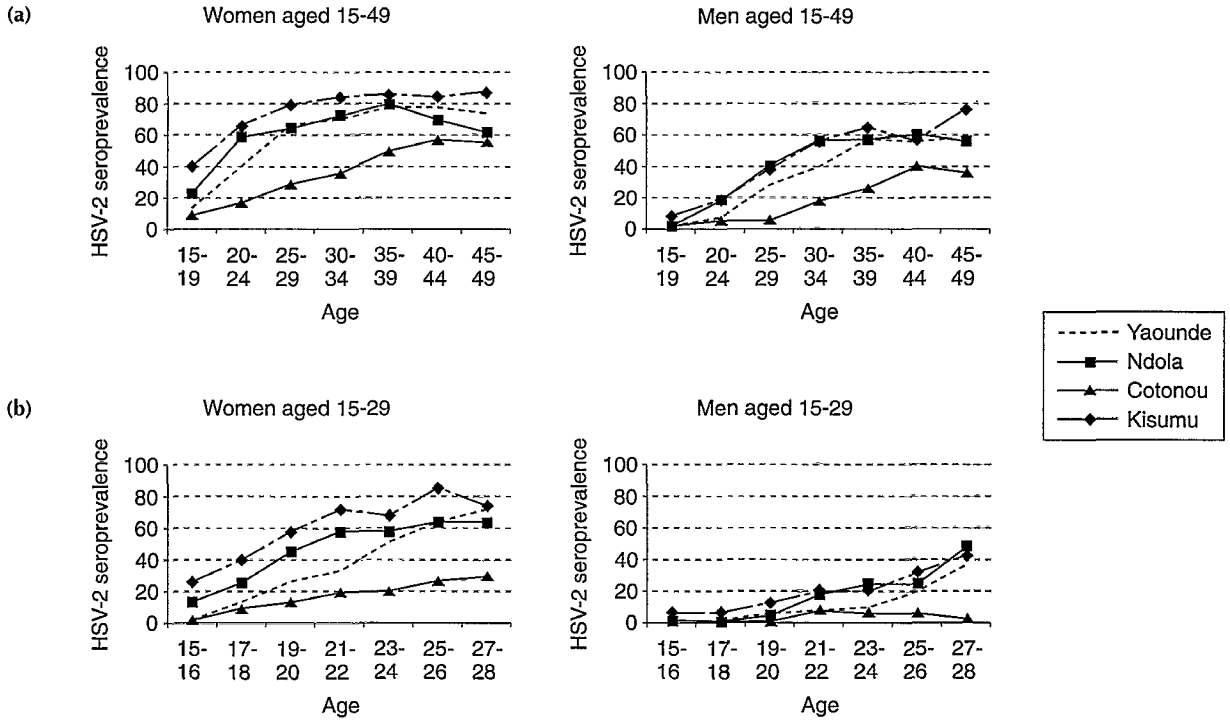

Fig. 1. Herpes simplex virus type-2 prevalence by city, sex and age group.

Results

Response rates for HSV-2 testing

The proportion of individuals tested for HSV-2 out of those eligible for the study varied from 583/1014 (57%) among men in Kisumu to 935/1139 (82%) among women in Cotonou, and the implications of non-response are discussed elsewhere [21]. In Yaoundé, Kisumu and Ndola, the response rate was substantially lower for men than women, and the main reason for non-response to the questionnaire was failure to contact potential study participants despite repeated visits to the home. The proportion of subjects tested for HSV-2 was lower than that tested for HIV, mainly because there was not always sufficient serum remaining to carry out HSV-2 testing. Overall, 919/1007 (91%) of subjects found to be HIV-positive were tested for HSV-2, compared with 5654/5890 (96%) of HIV-negative subjects.

Prevalence of HSV-2 infection by sex and age

HSV-2 prevalence was significantly higher in women than men in each city ($P < 0.001$). In women, overall prevalence ranged from 30% in Cotonou to 68% in Kisumu, and that in men from 12% in Cotonou to 36% in Ndola. There was a strong association of HSV-2 prevalence with age, generally rising rapidly between the ages of 15 and 29 before becoming stable (Fig. 1a and Table 1). In Cotonou, the increase in prevalence with age was less rapid, increasing steadily until age 40 in both men and women. Figure 1b shows prevalence by age among men and women aged younger than 30 in each city. The high prevalence among young women and men in Kisumu and Ndola is striking, with rates of up to 50% among those aged 15–24 years. Rates among young people in Yaounde were lower (27% among those aged 15–24), although prevalence increased steadily to reach 70% by age 30. The prevalence in Cotonou was substantially lower and increased steadily to 56% by age 40. Young men had generally lower prevalence, although again rates among those aged 15–24 were two- to three-fold higher in Kisumu and Ndola compared with Yaounde, and lower still in Cotonou.

Prevalence rates of HSV-1 infection among the random sample of 100 men and 100 women in each city were at least 96% in the age group 15–30 years. Due to this very high prevalence, no further analyses of HSV-1 were performed.

Univariate analysis: socio-demographic and behavioural factors

HSV-2 prevalence was lowest among never-married individuals, in all cities, and for both men and women. Prevalence was highest among those in polygamous relationships or those widowed, divorced or separated, with the strongest associations in Kisumu and Ndola for both men and women (Tables 2 and 3). Male circumcision is almost universal in Cotonou and Yaoundé, and there was little power to assess an association with HSV-2 in these cities. In Kisumu, 28% of men were circumcised, and the

Table 1. Herpes simplex virus type-2 prevalence by age, sex and city in four African cities

	Cotonou		Yaounde		Kisumu		Ndola	
Age (years)	Number positive/total	% (95% CI)	Number positive/total	% (95% CI)	Number positive/total	% (95% CI)	Number positive/total	% (95% CI)
Women								
15–19	18/200	9	34/238	14	83/213	39	51/221	23
20–24	34/195	17	90/232	39	121/184	66	124/214	58
25–29	55/189	29	126/184	68	119/151	79	107/167	64
30–34	41/114	36	92/131	70	92/109	84	80/111	72
35–39	47/94	50	70/90	78	60/70	86	66/82	80
40–44	39/68	57	60/77	78	41/49	84	35/51	69
45–49	42/75	56	37/50	74	42/48	88	24/39	62
Total	276/935	30 (27–34)	509/1002	51 (48–54)	558/824	68 (65–71)	487/885	55 (52–58)
Men								
15–19	2/172	1	5/189	3	12/142	8	1/106	1
20–24	11/203	5	15/207	7	25/145	17	23/133	17
25–29	9/179	5	47/161	29	30/79	38	49/117	42
30–34	22/132	17	47/118	40	46/80	58	57/103	55
35–39	22/82	27	54/92	59	38/58	66	35/61	57
40–44	18/43	42	37/67	55	29/50	58	30/49	61
45–49	19/52	37	31/53	58	22/29	76	21/38	55
Total	103/863	12 (10–14)	236/887	27 (24–30)	202/583	35 (32–40)	216/607	36 (32–40)

CI, Confidence interval.

risk of HSV-2 was significantly lower than among uncircumcised men [age-adjusted odds ratio (aOR) = 0.44, 95% CI = 0.3–0.7]. Circumcision is relatively rare in Ndola (8% of men), and rates of HSV-2 were similar in circumcised and uncircumcised men (41 and 35% respectively; Table 3).

Students were generally at lower risk than employed men and women, with the strongest protective effect in Ndola (women, aOR = 0.18, 95% CI = 0.1–0.4; men, aOR = 0.19, 95% CI = 0.04–0.8). Among women in Kisumu, prevalence was significantly lower among those with secondary level education compared with those with less than primary education (aOR = 0.49, 95% CI = 0.3–0.8; results not shown).

There was little association of religion and HSV-2, although Muslim men in Kisumu were at lower risk compared with other men (aOR = 0.44, 95% CI = 0.1–1.5). This lower risk was partly due to the confounding effect of circumcision, and after adjustment for circumcision status there was little effect of religion (adjusted odds ratio = 0.74, 95% CI = 0.2–2.8; results not shown).

After adjusting for current age, early age at first sexual intercourse was associated with a significantly increased risk of HSV-2 among women in all cities, and among men in Kisumu and Ndola (Tables 2 and 3). A small proportion of women who reported having not been sexually active were HSV-2-seropositive (4–11%), suggesting some under-reporting of sexual activity in women. Very few men who reported no sexual activity were positive (two each in Yaoundé and Kisumu). A strong association between HSV-2 and reported number of lifetime partners was seen consistently among women across the cities ($P < 0.001$). The association among men was strong in Yaoundé and Ndola, but weaker in Cotonou and Kisumu where men reporting over 20 partners were at only about twice the odds of men reporting one or two lifetime partners. There was also some association between HSV-2 and number of non-spousal partners in the past 12 months, and also with reported exchange of money for sex among women in Yaoundé and Ndola. Among men, a significant association with exchange of money for sex was seen in Cotonou only (Table 3).

Multivariate analysis: socio-demographic and behavioural factors

For each city, an initial multivariate model was fitted that included all of the aforementioned socio-demographic and behavioural variables significant at the 95% level for any city. Education and religion were not significant in the multivariate model for any city, and were excluded from the model. The final multivariate model included age group, marital status, circumcision status (men only),

Table 2. Herpes simplex virus type-2seroprevalence by socio-demographic and sexual behaviour factors in women, by city

	Cotonou		Yaoundé		Kisumu		Ndola	
	Positive (%)	OR (95% CI)[a]	Positive (%)	OR (95% CI)[a]	Positive (%)	OR (95% CI)[a]	Positive (%)	OR (95% CI)[a]
Marital status[b]	$P = 0.004$		$P = 0.32$		$P < 0.001$		$P < 0.001$	
Never married	44 (13%)	1	163 (35%)	1	75 (37%)	1	61 (25%)	1
Married, monogamous	107 (32%)	1.5 (0.9–2.4)	228 (62%)	0.94 (0.7–1.4)	284 (73%)	2.5 (1.7–3.9)	296 (62%)	2.5 (1.7–3.8)
Married polygamous	80 (53%)	2.7 (1.5–4.8)	44 (76%)	1.4 (0.7–2.8)	112 (84%)	4.8 (2.6–8.8)	14 (78%)	5.1 (1.5–16.6)
Widowed/divorced /separated	41 (47%)	2.1 (1.1–4.0)	73 (76%)	1.5 (0.8–2.6)	86 (90%)	6.0 (2.7–13.3)	106 (81%)	6.0 (3.3–10.9)
Age at first sex[c]	$P = 0.003$		$P = 0.06$		$P < 0.001$		$P = 0.007$	
< 15 years	19 (40%)	1	64 (58%)	1	181 (73%)	1	96 (63%)	1
15–17 years	97 (32%)	0.88 (0.4–1.7)	289 (54%)	0.94 (0.6–1.5)	281 (75%)	1.2 (0.8–1.8)	223 (59%)	0.95 (0.6–1.4)
18–19 years	96 (35%)	0.73 (0.4–1.4)	112 (59%)	0.83 (0.5–1.4)	64 (68%)	0.61 (0.3–1.1)	97 (63%)	0.89 (0.5–1.5)
≥ 20 years	58 (32%)	0.51 (0.3–1.0)	41 (57%)	0.61 (0.3–1.2)	26 (57%)	0.27 (0.1–0.5)	60 (60%)	0.62 (0.4–1.1)
Lifetime partners[c]	$P < 0.001$		$P < 0.001$		$P < 0.001$		$P < 0.001$	
None	5 (4%)	0.37 (0.1–1.1)	4 (4%)	0.36 (0.1–1.1)	6 (10%)	0.18 (0.1–0.5)	11 (11%)	0.37 (0.2–0.8)
1	53 (21%)	1	53 (30%)	1	75 (52%)	1	135 (42%)	1
2–4	189 (39%)	2.4 (1.7–3.5)	199 (49%)	2.3 (1.5–3.5)	410 (77%)	2.6 (1.7–3.9)	290 (73%)	3.5 (2.5–4.9)
5–9	25 (42%)	2.7 (1.5–5.1)	154 (73%)	5.5 (3.4–8.8)	61 (80%)	2.5 (1.3–5.0)	41 (79%)	4.9 (2.4–10.1)
≥ 10	2 (50%)	3.0 (0.4–24)	85 (79%)	7.1 (3.9–13.1)	5 (100%)		6 (100%)	
Number of partners in the past 12 months (excluding spouse)[c]	$P = 0.21$		$P < 0.001$		$P = 0.11$		$P < 0.001$	
None	233 (30%)	1	261 (48%)	1	455 (68%)	1	410 (53%)	1
1	34 (24%)	1.3 (0.8–2.1)	154 (49%)	1.7 (1.2–2.4)	81 (65%)	1.4 (0.9–2.2)	70 (67%)	2.4 (1.5–3.9)
> 1	7 (30%)	1.7 (0.6–4.6)	93 (66%)	3.6 (2.3–5.7)	21 (68%)	1.7 (0.7–3.9)	6 (60%)	3.2 (0.8–13.2)
Exchanged money for sex in past 12 months[b]	$P = 0.47$		$P < 0.001$		$P = 0.53$		$P < 0.001$	
No	269 (34%)	1	457 (54%)	1	513 (74%)	1	440 (60%)	1
Yes	1 (9%)	0.49 (0.1–4.1)	49 (78%)	3.6 (1.8–7.0)	39 (60%)	1.2 (0.7–2.1)	36 (69%)	3.3 (1.6–6.5)

[a] Age-adjusted odds ratio (OR) and 95% confidence interval (CI). [b] *P* values for heterogeneity in HSV-2 prevalence, adjusting for age. [c] *P* values for linear trend with HSV-2 prevalence, adjusting for age.

occupation, age at first sexual intercourse, number of lifetime partners, number of non-spousal partners in the past 12 months (women only), and exchange of money for sex in the past 12 months (men only). Results are presented in Tables 4 and 5.

The strong association between HSV-2 infection and age persisted after adjusting for other factors, with the strongest trend in Yaoundé for both women and men, where those aged older than 40 were at over nine times the odds of being HSV-2-seropositive as those aged younger than 20.

Table 3. Herpes simplex virus type-2 (HSV-2) seroprevalence by socio-demographic and sexual behaviour factors in men, by city

	Cotonou		Yaoundé		Kisumu		Ndola	
	Positive (%)	OR (95% CI)[a]	Positive (%)	OR (95% CI)[a]	Positive (%)	OR (95% CI)[a]	Positive (%)	OR (95% CI)[a]
Marital status[b]	$P = 0.01$		$P < 0.001$		$P < 0.001$		$P < 0.001$	
Never married	18 (4%)	1	73 (13%)	1	30 (11%)	1	30 (11%)	1
Married, monogamous	59 (21%)	2.9 (1.3–6.3)	140 (52%)	2.2 (1.4–3.4)	139 (53%)	3.6 (1.8–6.9)	156 (54%)	3.0 (1.7–5.4)
Married polygamous	22 (39%)	4.8 (1.8–12)	12 (60%)	2.6 (1.0–7.1)	23 (72%)	6.2 (2.2–18)	10 (59%)	4.0 (1.3–12)
Widowed/divorced/ separated	3 (19%)	2.7 (0.6–12)	11 (38%)	1.5 (0.6–3.4)	9 (64%)	6.7 (1.9–24)	20 (67%)	5.6 (2.3–14)
Circumcised[b]	$P = 0.51$		$P = 0.45$		$P < 0.001$		$P = 0.93$	
No	1 (10%)	1	1 (13%)	1	161 (38%)	1	194 (35%)	1
Yes	102 (12%)	2.0 (0.2–18)	235 (27%)	2.2 (0.2–21)	41 (26%)	0.4 (0.3–0.7)	22 (41%)	1.0 (0.5–1.9)
Age at first sex[c]	$P = 0.47$		$P = 0.43$		$P = 0.02$		$P = 0.01$	
< 15 years	10 (13%)	1	27 (19%)	1	60 (37%)	1	35 (34%)	1
15–17 years	34 (13%)	0.76 (0.3–1.8)	100 (26%)	1.0 (0.6–1.7)	85 (35%)	0.6 (0.3–0.9)	97 (42%)	1.2 (0.7–2.1)
18–19 years	27 (14%)	0.80 (0.3–1.9)	59 (35%)	0.88 (0.5–1.6)	33 (39%)	0.6 (0.3–1.0)	46 (42%)	0.94 (0.5–1.8)
≥ 20 years	32 (15%)	0.71 (0.3–1.6)	48 (41%)	0.84 (0.4–1.5)	21 (43%)	0.6 (0.3–1.2)	38 (37%)	0.66 (0.4–1.2)
Lifetime partners[c]	$P = 0.27$		$P = 0.001$		$P = 0.04$		$P < 0.001$	
None	0 (0%)	0	2 (3%)	4.0 (0.3–51)	2 (5%)	0.7 (0.1–3.5)	0 (0%)	0
1–2	9 (5%)	1	5 (6%)	1	17 (17%)	1	17 (14%)	1
3–4	20 (12%)	2.0 (0.8–4.6)	14 (16%)	2.1 (0.7–6.6)	34 (29%)	1.5 (0.7–3.1)	41 (31%)	1.9 (1.0–3.8)
5–9	39 (18%)	1.9 (0.9–4.3)	37 (22%)	3.0 (1.1–8.5)	70 (43%)	1.7 (0.9–3.3)	75 (48%)	3.4 (1.8–6.6)
10–19	18 (17%)	2.1 (0.8–5.0)	50 (28%)	3.0 (1.0–8.3)	48 (46%)	1.7 (0.8–3.5)	38 (55%)	5.0 (2.4–11)
≥ 20	16 (24%)	2.3 (0.9–5.9)	117 (44%)	5.2 (1.9–14)	27 (51%)	2.1 (0.9–4.8)	32 (68%)	7.7 (3.3–18)
Number of partners in the past 12 months (excluding spouse)[c]	$P = 0.23$		$P = 0.93$		$P = 0.30$		$P = 0.87$	
None	55 (12%)	1	67 (24%)	1	132 (44%)	1	150 (38%)	1
1	30 (13%)	1.3 (0.8–2.2)	48 (25%)	1.4 (0.8–2.3)	40 (24%)	0.7 (0.5–1.2)	35 (32%)	1.1 (0.7–1.9)
> 1	18 (12%)	1.9 (1.0–3.5)	120 (29%)	1.6 (1.0–2.4)	29 (25%)	0.8 (0.5–1.4)	31 (34%)	1.2 (0.7–2.2)
Exchanged money for sex in past 12 months[b]	$P = 0.004$		$P = 0.11$		$P = 0.55$		$P = 0.58$	
No	90 (11%)	1	185 (25%)	1	183 (35%)	1	190 (37%)	1
Yes	13 (23%)	3.2 (1.5–6.6)	51 (36%)	1.4 (0.9–2.2)	19 (33%)	1.2 (0.6–2.4)	26 (29%)	0.86 (0.5–1.5)

[a] Age-adjusted odds ratio (OR) and 95% confidence interval (CI). [b] *P* values for heterogeneity in HSV-2 prevalence, adjusting for age.
[c] *P* values for linear trend with HSV-2 prevalence, adjusting for age.

Table 4. Multivariate analysis of socio-demographic and behaviour factors for herpes simplex virus type-2 seropositivity in women, by city

	Cotonou (n = 935)	Yaoundé (n = 1003)	Kisumu (n = 824)	Ndola (n = 885)
Age group				
15–19 years	1	1	1	1
20–24 years	1.3 (0.6–2.6)	1.8 (1.1–3.0)	1.9 (1.1–3.1)	2.2 (1.3–3.6)
25–29 years	2.0 (0.9–4.3)	5.2 (2.9–9.4)	3.2 (1.8–5.8)	1.9 (1.1–3.4)
30–39 years	3.4 (1.6–7.5)	6.9 (3.7–13)	4.6 (2.5–8.6)	3.2 (1.8–5.8)
40–49 years	6.4 (2.8–15)	9.0 (4.4–18)	4.9 (2.3–11)	2.0 (1.1–4.0)
Marital status				
Never married	1	1	1	1
Married	2.3 (1.2–4.2)	1.2 (0.7–2.2)	2.1 (1.1–4.1)	2.7 (1.5–5.0)
Divorced/widowed/separated	2.1 (1.0–4.2)	1.0 (0.5–1.9)	3.4 (1.4–8.1)	4.0 (2.1–8.0)
Occupation				
Employed	1	1	1	1
Student	1.3 (0.7–2.5)	0.58 (0.3–1.0)	0.95 (0.4–2.2)	0.38 (0.2–0.9)
Homemaker	0.8 (0.5–1.3)	1.4 (1.0–2.1)	1.2 (0.8–1.9)	0.62 (0.4–0.9)
Other	1.1 (0.6–2.0)	0.88 (0.5–1.5)	1.2 (0.7–2.0)	0.70 (0.4–1.2)
Age at first sex				
< 15 years	1	1	1	1
15–17 years	0.94 (0.5–1.9)	1.00 (0.6–1.6)	1.3 (0.9–2.0)	1.2 (0.8–1.9)
18–19 years	0.82 (0.4–1.7)	0.97 (0.6–1.7)	0.76 (0.4–1.4)	1.6 (0.9–2.7)
≥ 20 years	0.68 (0.3–1.4)	0.97 (0.5–2.0)	0.38 (0.2–0.8)	1.1 (0.6–2.0)
Lifetime partners				
None	0.36 (0.1–1.3)	0.50 (0.1–1.7)	0.23 (0.1–0.7)	0.79 (0.3–2.0)
1	1	1	1	1
2–4	1.7 (1.2–2.5)	2.3 (1.6–3.4)	1.6 (1.1–2.4)	3.5 (2.3–5.4)
5–9	1.5 (0.8–2.9)	3.9 (2.5–6.0)	1.2 (0.6–2.4)	2.8 (1.3–6.2)
≥ 10	2.0 (0.2–18)	4.3 (2.3–8.0)		–
Number of non-spousal partners in past 12 months				
None	1	1	1	1
1	1.4 (0.7–2.5)	1.6 (0.9–2.7)	1.5 (0.8–2.7)	2.0 (1.1–3.9)
> 1	1.7 (0.5–5.1)	2.3 (1.2–4.3)	1.7 (0.6–4.6)	1.6 (0.3–7.7)

Data presented as odds ratios and 95% confidence intervals, adjusted for all variables in the Table.

Being currently or previously married was associated with at least a doubling of odds for women except in Yaoundé, where never-married women were at similar risk as ever-married women after adjusting for other factors. Among men, the strongest effect of marital status was seen among men in Kisumu and Ndola, where previously married men were at the highest risk [Kisumu, multiple adjusted OR (mOR) = 6.2, 95% CI = 1.6–23; Ndola, mOR = 4.5, 95% CI = 1.7–12.1; Table 5]. Male circumcision was significantly associated with a reduced risk of HSV-2 infection in Kisumu (mOR = 0.41, 95% CI = 0.3–0.7), but there was little evidence of an association in Ndola (mOR = 1.1, 95% CI = 0.5–2.2), although this was based on only 54 circumcised men. There was little association of HSV-2 prevalence with occupation after adjusting for other factors, except that, among women in Ndola, employed women were at higher risk than other women.

Age at first sexual intercourse was significantly associated with HSV-2 only among women in Kisumu, where women who reported sexual debut at age 20 or older were at lower risk than those with sexual debut before age 15 (mOR = 0.38, 95% CI = 0.2–0.8; Table 4). Increased number of lifetime partners was significantly associated with HSV-2 prevalence in each city among women (P value for trend < 0.003), with the strongest association in Yaoundé (where women with at least five lifetime partners were at over four times the odds of being HSV-2-positive compared with women with one partner). Women with more than one non-spousal partner in the past year were also at higher risk in each city, with the strongest association in Yaounde (mOR = 2.3, 95% CI = 1.2–4.3; Table 4). There was a significant trend with increasing number of non-spousal partners in the past year in Yaoundé and Ndola (P < 0.05). Among men there was a significant trend in HSV-2 risk with increas-

Table 5. Multivariate analysis for socio-demographic and behaviour factors associated with herpes simplex virus type-2 seropositivity in men, by city

	Cotonou (*n* = 863)	Yaoundé (*n* = 887)	Kisumu (*n* = 583)	Ndola (*n* = 607)
Age group				
15–19 years	1	1	1	1
20–24 years	1.3 (0.2–6.7)	1.7 (0.5–5.2)	1.6 (0.6–3.8)	4.8 (0.6–39)
25–29 years	0.59 (0.1–3.6)	6.4 (2.2–20)	2.7 (0.9–7.6)	8.5 (1.0–72)
30–39 years	1.9 (0.3–11)	11.1 (3.5–35)	5.3 (1.9–15)	9.8 (1.1–85)
40–49 years	4.2 (0.7–27)	10.9 (3.1–38)	5.0 (1.7–15)	9.4 (1.0–84)
Marital status				
Never married	1	1	1	1
Married	3.0 (1.4–6.5)	2.4 (1.5–3.8)	3.5 (1.7–7.0)	3.1 (1.6–5.9)
Divorced/widowed/separated	2.7 (0.6–12)	1.5 (0.6–3.5)	6.2 (1.6–23)	4.5 (1.7–12.1)
Circumcised				
No	-	-	1	1
Yes	-	-	0.41 (0.3–0.7)	1.1 (0.5–2.2)
Occupation				
Employed	1	1	1	1
Student	0.47 (0.1–1.6)	1.0 (0.5–1.9)	0.70 (0.3–1.9)	0.5 (0.1–2.5)
Other	1.3 (0.7–2.3)	0.66 (0.4–1.0)	0.63 (0.4–1.0)	1.4 (0.8–2.4)
Age at first sex				
< 15 years	1	1	1	1
15-17 years	0.80 (0.3–1.9)	1.0 (0.6–1.8)	0.56 (0.3–1.0)	1.2 (0.7–2.2)
18-19 years	0.85 (0.3–2.0)	1.0 (0.5–2.0)	0.64 (0.3–1.3)	1.3 (0.6–2.5)
≥ 20 years	0.89 (0.4–2.2)	1.2 (0.6–2.4)	0.61 (0.3–1.4)	1.3 (0.6–2.7)
Lifetime partners				
None	0	1.8 (0.3–12)	0.43 (0.1–2.2)	0
1–2	1	1	1	1
3–4	1.8 (0.7–4.3)	2.6 (0.8–8.5)	0.99 (0.5–2.2)	1.9 (0.9–4.0)
5–9	1.6 (0.5–3.7)	3.3 (1.1–9.6)	1.2 (0.6–2.6)	4.2 (2.0–8.5)
10–19	1.62 (0.6–4.3)	3.5 (1.2–10)	1.2 (0.5–2.8)	6.5 (2.8–15)
≥ 20	1.6 (0.6–4.5)	6.2 (2.2–18)	1.4 (0.5–3.7)	11 (4.1–28)
Exchange of money for sex in past 12 months				
No	1	1	1	1
Yes	3.2 (1.4–6.9)	1.4 (0.9–2.2)	1.5 (0.7–3.0)	0.48 (0.2–0.9)

Data presented as odds ratios and 95% confidence intervals, adjusted for all variables.

ing number of lifetime partners in Yaoundé and Ndola ($P < 0.001$), with men reporting more than 20 partners at over five times the odds of infection of men with one or two lifetime partners. In contrast, there was little evidence of association with number of lifetime partners among men in Cotonou and Kisumu (Table 5). Reported exchange of money for sex in the past 12 months was associated with a significantly increased risk of infection among men in Cotonou (mOR = 3.2, 95% CI = 1.4–6.9), but with a decreased risk in Ndola (mOR = 0.48, 95% CI = 0.2–0.9).

Association of HSV-2 infection with other sexually transmitted infections

After adjusting for the socio-demographic and behavioural factors shown in Tables 4 and 5, there was a strong and consistent association between HIV infection and HSV-2 infection. Among women, the association between HSV-2 and HIV infection was a four- to five-fold increase in odds and highly statistically significant in each city. Similar associations were seen in men, with the strongest association in Kisumu (mOR = 7.9, 95% CI = 4.1–15.4; Table 6).

Table 6. Association of herpes simplex virus type-2 with other sexually transmitted infections, adjusted for socio-demographic and behavioural factors by sex and city

	Women				Men			
	Cotonou	Yaoundé	Kisumu	Ndola	Cotonou	Yaoundé	Kisumu	Ndola
HIV status								
Negative	1	1	1	1	1	1	1	1
Positive	5.4 (2.1–14)	5.5 (1.7–18)	4.0 (2.0–8.0)	4.5 (2.6–7.7)	5.5 (2.1–15)	5.2 (2.2–13)	7.9 (4.1–15)	4.6 (2.7–7.7)
Syphilis								
No	1	1	1	1	1	1	1	1
Yes	0.53 (0.1–2.5)	4.4 (1.4–14)	–[a]	4.3 (1.8–10)	1.6 (0.4–6.6)	1.7 (0.9–3.3)	0.67 (0.2–2.6)	2.0 (1.0–3.8)
Gonorrhoea								
No	1	1	1	1	1	1	1	1
Yes	9.4 (1.6–55)	2.7 (0.8–8.5)	0.99 (0.1–11)	6.1 (0.6–63)	1.6 (0.3–8.7)	3.9 (0.9–17)	–[c]	1.6 (0.1–44)
Chlamydia								
No	1	1	1	1	1	1	1	1
Yes	1.2 (0.3–5.5)	1.2 (0.6–2.4)	0.93 (0.3–3.0)	6.1 (1.6–24)	1.2 (0.3–5.9)	0.67 (0.3–1.5)	0.43 (0.1–2.8)	1.9 (0.4–8.7)
Trichomonas vaginalis								
No	1	1	1	1	–	–	–	–
Yes	2.1 (0.9–5.0)	2.0 (1.2–3.5)	1.0 (0.5–1.9)	1.4 (0.8–2.4)				
History of STD in past 12 months								
No	–[b]	–	–	–	1	1	1	1
Yes					0.95 (0.5–2.0)	1.1 (0.7–1.7)	1.5 (0.8–3.0)	2.6 (1.4–4.9)

Data presented as odds ratios and 95% confidence intervals. [a] Among women in Kisumu, there were 32 cases of syphilis, and 29 (91%) of these were HSV-2-positive. It was not possible to estimate the odds ratio due to confounding with HIV (the three women with syphilis who were HSV-2-negative were also HIV-negative). [b] Women were not asked about history of sexually transmitted diseases (STDs). [c] There were no cases of gonorrhoea among men in Kisumu.

Syphilis was significantly associated with HSV-2 infection among women in Yaoundé and Ndola, and to a lesser extent among men in these two cities. The numbers of cases of non-ulcerative sexually transmitted infections were generally small and confidence intervals were wide. However, the magnitude of association between HSV-2 and gonorrhoea was large among women in Cotonou and Ndola (Cotonou, mOR = 9.4, 95% CI = 1.6–55; Ndola, mOR = 6.1, 95% CI = 0.6–63), and to a lesser extent among men in Yaoundé (mOR = 3.9, 95% CI = 0.9–17). There was a significant association between chlamydial infection and HSV-2 among women in Ndola (mOR = 6.1, 95% CI = 1.6–24), but not in other cities. *Trichomonas vaginalis* infection was associated with a twofold odds ratio of HSV-2 infection among women in Cotonou and Yaoundé. Among men, a reported history of symptoms suggestive of a sexually transmitted infection in the past 12 months was associated with a significantly higher risk of HSV-2 in Ndola (mOR = 2.6, 95% CI = 1.4–4.9), and to a lesser extent in Kisumu (mOR = 1.5, 95% CI = 0.8–3.0). For each city and both sexes, there was little change in the associations between HSV-2 and socio-demographic or behavioural factors shown in Tables 4 and 5 after adjustment for these other sexually transmitted infections.

Discussion

The high seroprevalences of HSV-2 in these cities are consistent with results from other African studies in rural populations [9–11], antenatal clinic attenders [22], trucking company workers [13], factory workers [12,23], STD patients [14,15,17] and commercial sex workers [16]. In addition, a collaborative study from 1985 reported HSV-2 prevalence in adults ranging from 27% in rural Rwanda to 71% in Brazzaville, Congo [7], although sex-specific prevalences were not reported.

The higher prevalence among women has been observed in other population-based studies worldwide [9,11,24–30]. This may be partly due to the greater mucosal surface of the female genital tract, resulting in higher male-to-female transmission risk per exposure [11].

In this study, the factors consistently and independently associated with HSV-2 infection across the four cities in both men and women were older age, being married, and HIV infection. Prevalence of HSV-2 was highest in Kisumu, followed by Ndola and Yaoundé, and lowest in Cotonou. In Kisumu and Ndola, the prevalence of HSV-2 infection is particularly high in the younger age groups, which is probably in part due to the younger

average age at marriage in these cities, and also due to high levels of concomitant HIV infection in male partners [31], which may enhance the HSV-2 infectivity of those partners. The situation in Yaoundé differs, with a much lower prevalence of HIV infection (8% in women and 4% in men) but the highest rates of partner change. In Yaoundé, the number of lifetime and recent partners was strongly associated with HSV-2 prevalence, suggesting that it may be the rates of partner change in Yaoundé that are primarily responsible for the sharp increase in HSV-2 prevalence at age 20–29.

The increased risk among married individuals is probably due to increased exposure to infection among individuals married to a HSV-2-positive partner, and more frequent sexual contact in marriage, and is similar to results seen for HIV infection [32]. The significant trend between HSV-2 and lifetime number of partners is consistent with previous data from African populations [9,11], and some US and European populations [24,25,29,30,33]. This trend supports the use of HSV-2 seroincidence as a biological, sensitive and affordable tool in evaluating behaviour change in HIV/STD prevention interventions, especially among women.

Several factors are likely to have contributed to the consistently strong association between HSV-2 and HIV infections in these populations. As both infections are sexually transmitted, it is possible that the association is partly due to residual confounding by unmeasured sexual behaviour. However, HSV-2 infection is likely to increase susceptibility to HIV because of the lesions it causes that are not always recognized by the infected individual [4,34]. It is also possible that HIV infection increases susceptibility to HSV-2 infection, and this biological synergy may explain the very strong associations seen. Epidemiological evidence for the role of HSV-2 infection in increasing susceptibility to HIV comes from several cohort studies of heterosexual acquisition of HIV [8,13,23,35] from Zimbabwe, Kenya and Thailand. These four studies found that HSV-2 infection at baseline was associated with an increased risk of HIV acquisition: unadjusted rate ratio (RR) = 1.4, $P = 0.06$ in the Zimbabwe study; unadjusted RR = 2.9, 95% CI = 1.2–7.0 in the Kenyan study; and adjusted RR = 3.1 and 2.0 in the Thai studies (although the latter was based on only 14 incident HIV cases and was not statistically significant). There have also been several prospective studies of HSV-2 infection and male-to-male transmission of HIV [4,6,36,37]. Three of these studies [4,6,37] found that HSV-2 infection was significantly associated with increased HIV acquisition, with unadjusted odds ratios from 2.2 to 6.0. Two of these studies [4,37] additionally adjusted for sexual behaviour and then found no association between HSV-2 and HIV (matched odds ratio = 1.0). Evidence for the casual role of HIV infection in increasing HSV-2 susceptibility comes from the prospective study in Zimbabwe [23], which found a significantly increased risk of HSV-2 incidence among men who were HIV-seropositive at baseline (adjusted hazard ratio = 4.7, 95% CI = 3.3–6.7).

At a population level, HSV-2 may be a driving force in the HIV epidemics if it also increases HIV infectivity among dually infected individuals. This is likely to happen because HSV-2 genital ulcers have been shown to facilitate HIV shedding [38]. There is currently little epidemiological evidence that non-ulcerative HSV-2 infection increases HIV genital shedding [39], although there is evidence that co-infection of CD4 cells with HIV-1 and HSV-2 accelerates HIV-1 replication through the HSV regulatory protein ICP4 (but not HSV-2 replication) [40,41]. Conversely, HIV infection may increase transmission of HSV-2 both by increasing prevalence and severity of HSV-2 genital ulcers due to immunodeficiency. Moreover, it has been shown that HIV infection enhances the genital shedding of HSV-2 even in the absence of clinical lesions [39,42,43]. Thus, available evidence so far suggests that epidemics of HIV and HSV-2 may be reinforcing each other, which would be consistent with the strong association between these two infections seen in this study both at the individual and population levels.

The test used in this study to detect antibodies against HSV-2 was found to have a high sensitivity of 93% and a specificity of 96%. However, it has been suggested that the sensitivity of similar assays may be lower in AIDS patients [44]. The effect of this would be to under-estimate the strength of association between HIV and HSV-2 in our data. Furthermore, the HSV-2 antibodies are thought to persist lifelong, but a recent study of reproducibility of a glycoprotein G assay in a cohort study showed that 15% of subjects initially seropositive were found to be seronegative when serum was re-tested [45]. Again, the limitations of the assay should not affect our interpretation, given the strength and consistency of the association between HSV-2 and HIV seen in our data. Lack of sensitivity suggests that, if anything, HSV-2 prevalence is under-estimated in this study.

Response rates for HIV and HSV-2 testing were as low as 57% of the eligible men in Kisumu, and detailed discussion of the possible effects of selection bias is considered elsewhere [21]. For the present analysis, the main concern is that substantially fewer HIV-positive subjects than HIV-negative subjects were tested for HSV-2. Given the association between HSV-2 and HIV infections, the effect of this would be to under-estimate HSV-2 prevalence in the general populations from which our study population was selected. It is also likely that the risk profile of the individuals for whom we have HSV-2 data is not entirely representative of the general population, yet this should not affect the magnitude of the associations seen within our data.

To conclude, these data have confirmed a strong and consistent association between HSV-2 and HIV infection, after adjusting for reported sexual behaviour and other confounding factors. This is consistent with results from epidemiological studies in other populations, as well as clinical studies of genital shedding, and suggests that HSV-2 infection may play an important role in the spread of HIV infection in sub-Saharan Africa. At a population level, the prevalence of HSV-2 infection was generally higher in the cities where the spread of HIV has been more rapid. However, the question remains about the role HSV-2 infection plays in explaining the differences in spread of HIV between Kisumu and Ndola, on the one hand, and Cotonou and Yaoundé, on the other. The high prevalence of HSV-2 in Kisumu and Ndola may partly be the result of the high HIV prevalence and younger age at marriage, and future studies would include analysis of stored serum from community surveys to track HSV-2 prevalence from before the HIV epidemic took hold. It might be possible that, in the initial stages of the HIV epidemics in these cities, factors other than HSV-2 (such as lack of male circumcision) also played an important role in the spread of HIV but that, once HIV infection reached a certain level, both epidemics fuelled each other and the role of HSV-2 became more important.

The association between HSV-2 and HIV infections has major public health implications for sub-Saharan Africa. Suppressive antiviral therapy for HSV-2 infection is not feasible in general populations in most developing countries, and the high prevalence of HSV-2 may limit the effectiveness of high levels of antibiotic treatment of STDs on HIV transmission. The high prevalence of both infections among young people underlines the urgent need for increased education and counselling among adolescents to discourage unprotected sexual contact and reduce the number of sexual partners. However, it seems that the best hope for widespread control of HSV-2 infection in many countries lies in a vaccine to prevent genital herpes [46].

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