HIV incidence and prevalence in Bloemfontein and Rustenburg, South Africa: a prospective study

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HIV incidence and prevalence in Bloemfontein and Rustenburg, South Africa: a prospective study

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ARTICLE SUMMARY

Article Focus

- Primary objective: measure HIV incidence and prevalence in two prospective cohorts of HIV-negative women
- Secondary objective 1: measure pregnancy rates
- Secondary objective 2: measure retention rates

Key Messages

- The project aimed to measure the HIV burden in two smaller South African cities, and to prepare new clinical research groups there for future HIV prevention studies.
- HIV seroprevalence was 21.2% in Bloemfontein and 23.5% in Rustenburg.
- HIV incidence rates were 5.5 per 100 person-years (PY) in Bloemfontein and 3.0 per 100 PY in Rustenburg.

Study Strengths

Study strengths include the longitudinal design with high retention rates. Classification of the HIV outcome was highly accurate, using multiple rapid tests confirmed by later PCR testing. Testing of stored baseline specimens allowed us to pinpoint seroconversion and rule out acute pre-study infections. And direct prospective measurement of HIV infection circumvented the lingering issues with the accuracy of cross-sectional incidence estimation.

Study Limitations

The main study limitation is the validity of self-reported sexual behaviors, both for purposes of determining eligibility as well as for assessing risk during follow-up. Although we attempted to keep the eligibility factors secret from participants and outreach staff, we do not know if volunteers (both eligible and ineligible) guessed those factors and shared them with other women in the community.

A second weakness is that there were too few seroconversion events to do multivariable Cox regression analysis, and our univariable regression results for incident HIV infections were necessarily inconclusive. A limitation of the analysis of risk factors for prevalent HIV infections is that the behaviors reported at screening may not have reflected behaviors current at the time of infection. Finally, the lack of STI diagnostic testing precluded evaluating associations between HIV and other infections.

ABSTRACT

Objectives: The primary objective was to measure HIV incidence in two prospective cohorts of HIV-negative women. Secondary objectives included measuring pregnancy rates and participant retention rates.

Design: Cross-sectional HIV screening, followed by a prospective study following initially HIV-negative women for up to 6 monthly visits.

Setting: Primary health care clinics established for research purposes in Bloemfontein and Rustenburg, South Africa.

Participants: We enrolled women 18-35 years old and presumed at higher risk of sexual acquisition of HIV as indicated by self-reported sexual behavior or recent sexually transmitted infection symptoms. In Bloemfontein, cross-sectional screening enrolled 1364 women; 1154 were eligible for testing; 1145 agreed to be tested. The prospective study enrolled 401 HIV-negative women from screening. In Rustenburg, cross-sectional screening enrolled 946 women; 540 were eligible and underwent testing; 223 HIV-negative women entered the prospective study.

Primary and secondary outcomes: Baseline prevalences of HIV infection, and HIV incidence rates in the prospective cohorts, according to a double rapid test algorithm with a third rapid test for discrepant or indeterminate results. Pregnancy prevalences and pregnancy incidence rate in Bloemfontein. Participant retention rates in the prospective cohort until study end.

Results: In Bloemfontein, 1145 women were screened; 391 entered follow-up; 92.3% of participants completed 6 study visits. In Rustenburg, 540 women were screened; 194 entered follow-up; retention up to the point of early study termination was 88.6%. Overall HIV prevalence was 21.2% (95% confidence interval [CI] 18.9-23.6) in Bloemfontein and 23.5% (95% CI 19.9-27.1) in Rustenburg. Overall HIV incidence was 5.5 per 100 person-years (PY; 95% CI 2.5-10.4) in Bloemfontein and 3.0 per 100 PY (95% CI 0.4-10.8) in Rustenburg. Cross-sectional pregnancy prevalences were 6.5% in Bloemfontein and 8.6% in Rustenburg.

Conclusions: We observed substantial HIV incidence rates in both cohorts. Vigorous prevention efforts are needed in these cities.

INTRODUCTION

HIV/AIDS continues to exact a massive toll on the health, economics, and political infrastructure of communities around the world. In sub-Saharan Africa, the most heavily affected region, an estimated 22.4 million people were reported to be living with HIV in 2008 (1). While recent prevention trials assessing male circumcision, antiretroviral gel use and pre-exposure prophylaxis have shown significant promise (2-5), studies to corroborate these results and to evaluate new prevention methods will be required. To meet the ongoing need for research sites in countries with substantial HIV burden, FHI 360's Site Identification and Development Initiative (SIDI) undertook the development of previously research naïve sites in six countries to conduct future HIV prevention research.

The SIDI team partnered with groups in two South African cities for clinical research development: Bloemfontein in the Motheo District of Free State Province, and Rustenburg in the Bojanala District of Northwest Province. District-level HIV prevalence rates among antenatal women aged 15-49 were 27.8% and 34.9%, respectively in 2009; provincial HIV prevalence rates were 30.1% (Free State) and 30% (Northwest) (6). HIV incidence rates for both sites had not been measured.

The goal of the SIDI project was to develop HIV prevention research capacity and to determine the suitability of each site to implement future HIV prevention clinical trials. Each site conducted its own prevalence and incidence study using a similar protocol. The primary objective of each study was to measure HIV incidence in a prospective cohort of HIV-negative women at each study site. Secondary objectives included measuring pregnancy rates and participant accrual and retention rates in the cohort.

METHODS

Study Design and Recruitment

The study comprised cross-sectional screening with HIV testing, followed by a prospective cohort study with up to six monthly follow-up visits. In Bloemfontein, the study was conducted at the JOSHA Research Centre. Bloemfontein and the surrounding Mangaung municipality comprise a population of approximately 600,000 people. JOSHA consists of a general practice and an independent clinical research facility and can draw participants from the general practice as well as from cooperating government clinics. Participants for the cross-sectional screening were recruited using methods developed based on feedback from community engagement

activities led by the Mangaung-University Community Partnership Program (MUCPP) and input from key stakeholders in the community. Recruitment methods included: 1) recruitment from HIV counseling and testing, sexually transmitted disease, and family planning clinics; 2) targeted outreach activities in the community; and 3) referrals from other clinics. Participants for the prospective study were recruited from among the HIV-negative participants in cross-sectional screening.

In Rustenburg, with a population estimated at 350,000 in the 2001 census but which has grown considerably since then, the study was conducted at The Aurum Institute's Rustenburg Research Centre located downtown. The Rustenburg economy is largely driven by the platinum mining industry, and is home to a large, diverse and growing influx of residents attracted to the area for work in the mining industry. The Aurum Institute (formerly the Aurum Institute for Health Research) is a non-profit research organization that began activities in March 2007 to prepare the Rustenburg Municipality to engage in epidemiologic studies and future clinical research trials. Participants for the cross-sectional screening were recruited from a variety of clinic-based and community settings which included an emphasis on local colleges. To facilitate recruitment and enrollment for cross-sectional screening, the protocol was amended twice late in the study to add respondent driven sampling (RDS) as a recruitment method (7), and to liberalize the sexual behavior eligibility criterion. Both men and women were recruited for cross-sectional screening in Rustenburg, but only HIV-negative women were invited to participate in the prospective study as there was a separate male cohort study there.

Study Procedures

Following the informed consent process and assignment of a participant number, volunteers were considered enrolled. Enrolled participants were screened for eligibility for cross-sectional testing using a demographic and HIV risk factor questionnaire. Recruitment and screening staff at both sites were blind to the specific behavioral eligibility criteria to avoid the spread of information through the community, and to prevent volunteers from altering self-reported behaviors to gain access to the study. All participants were asked the full questionnaire regardless of their initial answers.

Only eligible women were tested. Eligibility for HIV and pregnancy testing was determined by age (18-35 at the time of screening) and presumed higher risk of sexual acquisition of HIV as defined by at least one of the following self-reported criteria in the past three months: 1)

treatment for, diagnosis of, or signs/symptoms of a sexually transmitted infection (STI); 2) vaginal or anal intercourse with more than one partner (unprotected intercourse in Rustenburg only); 3) vaginal or anal intercourse with a new sexual partner (unprotected intercourse in Rustenburg only); or 4) sex with an HIV-infected partner who is not using antiretroviral therapy (ART). Near the end of the Rustenburg study, we revised these criteria to remove the requirements for multiple partners and unprotected sexual acts for women, as we suspected that we were screening out many higher-risk women, and that condom use reporting was not accurate. Participants deemed eligible underwent HIV testing within the context of HIV pre- and post-test counseling in accordance with national guidelines. Women also had urine pregnancy tests. Results of both tests were provided to participants during their visits.

Women were eligible to enter the prospective cohort study if they met the cross-sectional age and behavioral criteria, were HIV- and pregnancy-negative, and were not planning to relocate from the area in the near future. The target prospective study size was 400 women at each site, followed monthly for up to 6 months. At each monthly visit, participants provided updated medical and behavioral information, underwent HIV and pregnancy testing, received syndromic management for STIs (as needed), were counseled on HIV risk reduction practices, and received free condoms.

HIV testing was performed on finger prick blood samples using a double rapid test algorithm with the Abbott Determine HIV – 1/2™ and Trinity Biotech Uni-Gold™ run in parallel. Two positive results were deemed positive and two negative results were deemed negative, which exceeded national HIV testing guidelines. Discrepant or indeterminate samples were tested with a third rapid test, SD BIOLINE HIV – 1/2 3.0, as a tie-breaker. Pregnancy testing was performed on urine samples using the Quidel QuickVue hCG-Combo test. All tests were run according to manufacturers' specifications.

Participants who tested HIV-positive at screening or during the cohort study were referred for care in accordance with local guidelines. In addition, those participants who seroconverted during the prospective phase were intensely counseled about secondary HIV prevention in the acute infection period, and in Rustenburg were invited to join a seroconvertor cohort with continued close monitoring. Participants who were found to be pregnant were referred for antenatal care. Participants who became pregnant during follow-up continued to make study visits.

Ethical Considerations

The protocols, informed consent forms, participant education and recruitment materials, and case report forms were reviewed and approved by the FHI ethics committee and the local ethics committees, the University of Free State ethics committee for Bloemfontein and the University of KwaZulu Natal Biomedical Research Ethics Committee for Rustenburg.

We obtained written informed consent from each participant prior to enrollment and data collection. Written consent was also obtained for long-term specimen storage and possible future testing, although consent for specimen storage was not required for study participation. Informed consent forms were available in English and were translated into local languages, Sesotho (Bloemfontein) and Setswana and IsiXhosa (Rustenburg) to enhance comprehension. Volunteers had their choice of language for the informed consent process, which was witnessed and verified by a non-study staff person for illiterate or low-literate participants.

Statistical Analysis

All eligible participants were included in the data analysis. For each site, we summarized participants' socio-demographic characteristics, self-reported STI symptoms and HIV risk behaviors at baseline for cross-sectional screening participants, and those women who entered the prospective study. We calculated baseline prevalences of HIV infection and pregnancy among women, along with 95% confidence intervals (CI). We used univariate and multivariable logistic regression analysis to evaluate the association between risk factors and prevalent HIV infection.

The incidence rate was calculated as the number of post-enrollment HIV seroconversions divided by the person-years (PY) of observation accumulated by the cohort. We calculated 95% CI using exact methods under the assumption that the number of HIV infections follows a Poisson distribution. We used proportional hazards regression to evaluate the associations between incident HIV infection and possible HIV risk factors including time-dependent participant characteristics, and time-varying risk behaviors and self-reported STI symptoms. Due to the small number of HIV seroconversions, we did univariate proportional hazards regression only. Data analyses were performed using SAS version 9.2 (Cary, North Carolina).

RESULTS

Eligible populations

In Bloemfontein, the cross-sectional screening enrolled 1364 women between February and October 2009. Of the 1364 enrolled women, 1154 were determined to be eligible for testing, and 1145 (84% of enrollees) chose to be tested in cross-sectional screening. The prospective study enrolled 401 HIV-negative women volunteers from screening. Of those 401 women, 399 were determined to be eligible, and 391 entered follow-up and were tested. Retention in the prospective study was excellent, with 92.3% of participants completing 6 study visits with a median 5.6 person-months of observation and a total 164.4 person-years.

In Rustenburg, cross-sectional screening enrolled 946 women and 968 men between November 2008 and December 2009. Data on the male participants are not presented here. Of the 946 enrolled women, 540 (57%) were eligible and underwent testing in cross-sectional screening; most of the women who were screened out from cross-sectional testing did not satisfy the behavioral or STI risk criteria. The prospective study enrolled 223 HIV-negative women volunteers, of whom 194 yielded HIV test data. Completion of follow-up in the prospective study was hindered when it was ended prematurely due to funding shortfalls, but retention in follow-up up to the point of study termination was 88.6% with a median 4.1 person-months of observation and a total 67.1 person-years.

Participant characteristics

Large percentages of participants in cross-sectional screening at both sites were ≤21 years, had a high school level education, were not employed, were unmarried, and lived with family members (Table 1). About one-third of the women were using an effective contraceptive at each site (hormonal method or IUD: 38.3% in Bloemfontein and 32.9% in Rustenburg; Table 1). STI testing was not done; self-reported STI signs/symptoms at baseline ranged from 2-15% prevalence in Bloemfontein, and from 22-37% in Rustenburg.

At baseline, women in the Bloemfontein study self-reported more risk behaviors than women in Rustenburg (Table 1). Two-thirds of women in Bloemfontein reported vaginal intercourse with two or more partners in the month preceding cross-sectional screening; the corresponding percentage in Rustenburg was 14%. Over half of the women in Bloemfontein reported having vaginal intercourse with their primary partners without a condom in the previous week, whereas

about a quarter of women in Rustenburg reported unprotected intercourse with primary partners in the previous week. Women in Bloemfontein were also more likely to report unprotected intercourse with a non-primary partner.

Table 1. Participant Features (N & %) of Eligible Women at Inception of Cross-sectional Screening and Prospective Study in Bloemfontein and Rustenburg Sites

	Bloemfontein		Rustenburg	
	Cross-sectional N = 1154	Prospective N = 399	Cross-sectional N = 540	Prospective N = 223
Age				
<21	520 (45.1)	206 (51.6)	239 (44.2)	115 (51.6)
22-24	259 (22.4)	92 (23.1)	130 (24.1)	53 (23.8)
25-31	284 (24.6)	76 (19.1)	138 (25.6)	46 (20.6)
>31	91 (7.9)	25 (6.3)	33 (6.1)	9 (4.0)
Education				
None - grade 5	32 (2.8)	7 (1.8)	4 (0.8)	0 (0.0)
Grade 6-9	181 (15.7)	49 (12.3)	27 (5.0)	10 (4.5)
Grade 10-12	860 (74.5)	333 (83.5)	454 (84.1)	188 (84.3)
Univ/college/grad	81 (7.0)	10 (2.5)	55 (10.2)	25 (11.2)
Employment				
No, unemployed	1000 (86.7)	352 (88.2)	463 (85.7)	196 (87.9)
Yes, part-time	78 (6.8)	25 (6.3)	31 (5.7)	11 (4.9)
Yes, full-time	76 (6.6)	22 (5.5)	46 (8.5)	16 (7.2)
Whom you live with				
Alone	45 (3.9)	12 (3.0)	25 (4.6)	8 (3.6)
Spouse	52 (4.5)	15 (3.8)	15 (2.8)	8 (3.6)
Family/relatives	859 (74.4)	315 (78.9)	375 (69.4)	160 (71.8)
Roommate/friend	103 (8.9)	34 (8.5)	28 (5.2)	12 (5.4)
Partner	95 (8.2)	23 (5.8)	97 (18.0)	35 (15.7)
Married				
No	1056 (91.5)	369 (92.5)	507 (93.9)	211 (94.6)
Yes	98 (8.5)	30 (7.5)	33 (6.1)	12 (5.4)

	Bloemfontein		Rustenburg	
	Cross-sectional N = 1154	Prospective N = 399	Cross-sectional N = 540	Prospective N = 223
Contraceptive use				
None	540 (46.9)	182 (45.6)	157 (29.1)	58 (26.0)
Oral	55 (4.8)	20 (5.0)	24 (4.4)	19 (8.5)
Injectable	384 (33.3)	138 (34.6)	132 (24.4)	65 (29.2)
IUD	2 (0.2)	0 (0.0)	1 (0.2)	1 (0.5)
Condoms	148 (12.9)	52 (13.0)	201 (37.2)	80 (35.9)
Other	23 (2.0)	7 (1.8)	1 (0.2)	0 (0.0)
Missing	2 (0.2)	0 (0.0)	24 (4.4)	0 (0.0)
STI signs/symptoms				
Vaginal discharge	155 (13.4)	52 (13.0)	154 (28.5)	59 (26.5)
Painful urination	103 (8.9)	31 (7.8)	153 (28.3)	57 (25.6)
Lower abd. pain	115 (10.0)	39 (9.8)	202 (37.4)	82 (36.8)
Vaginal itching	170 (14.7)	60 (15.0)	155 (28.7)	63 (28.5)
Dyspareunia	104 (9.0)	35 (8.8)	121 (22.4)	50 (22.4)
Vaginal sore	27 (2.3)	12 (3.0)	142 (26.3)	66 (29.6)
No. sex partners in last month				
0	53 (4.6)	18 (4.5)	85 (15.7)	40 (17.9)
1	362 (31.4)	120 (30.1)	400 (74.1)	155 (69.5)
2+	739 (64.0)	261 (65.4)	55 (10.2)	28 (12.6)
Vaginal sex w/o condom past week w primary partner	638 (55.3)	202 (50.6)	190 (35.2)	78 (35.0)
Vaginal sex w/o condom past week w other partner	150 (13.0)	50 (12.5)	8 (1.5)	3 (1.3)

The baseline features of women who entered the prospective studies were broadly similar to those of the source screening populations at both sites (Table 1), and there was little indication of selection for a particular subgroup into the prospective cohort.

HIV prevalence

In Bloemfontein, the HIV prevalence in cross-sectional screening was 21.2% (95% confidence interval [CI] 18.9-23.6) overall: 13.4% in those 18-24 years; 38.1% in those 25-29 years; and 37.1% in women aged 30-35 years (Table 2). In Rustenburg, the overall prevalence was 23.5%

(95% CI 19.9-27.1) among women, ranging from 18.7% in those age 18-24 to 31.8% in those age 25-29 to 37.7% in those age 30-35 (Table 2).

Table 2. HIV Prevalence among Women in Cross-sectional Screening by Age Group and Research Site

	Bloemfontein	Rustenburg
Age 18-24		
Eligible Population[1], N	776	369
HIV positive, N	104	69
Prevalence (95% CI[2])	13.4% (11.0-15.8)	18.7% (14.7-22.7)
Age 25-29		
Eligible Population[1], N	226	110
HIV positive, N	86	35
Prevalence (95% CI[2])	38.1% (31.7-44.4)	31.8% (23.1-40.5)
Age 30+		
Eligible Population[1], N	143	61
HIV positive, N	53	23
Prevalence (95% CI[2])	37.1% (29.1-45.0)	37.7% (25.5-49.9)
TOTAL		
Eligible Population[1], N	1145	540
HIV positive, N	243	127
Prevalence (95% CI[2])	21.2% (18.9- 23.6)	23.5% (19.9- 27.1)

[1] Eligible and had HIV test result
[2] Approximate confidence interval

Factors associated with prevalent HIV

Three baseline factors were significantly associated with prevalent HIV infection at both study sites (Table 3): age 30 or older compared with 18-24; less versus more educational attainment; and living with a child versus not living with child. Self-reported signs or symptoms of STI were associated with HIV in Bloemfontein only, and non-use of contraception versus use of condoms was associated with HIV in Rustenburg only. Sexual behavior factors were not significantly associated with prevalent HIV infection.

Table 3. Multivariable Logistic Regression Analysis of Factors Associated with Prevalent HIV among Women at the Bloemfontein and Rustenburg Sites

	Bloemfontein			Rustenburg		
Factor	**Odds Ratio**	**95% CI**		**Odds Ratio**	**95% CI**	
Age 18-24 vs 30+	**0.389**	**0.243**	**0.623**	**0.485**	**0.284**	**0.826**
Age 24-29 vs 30+	1.222	0.766	1.950	0.844	0.502	1.419
Education: Less than High school	**2.240**	**1.552**	**3.233**	**1.979**	**1.099**	**3.563**
Employed: yes	0.913	0.587	1.419	0.790	0.509	1.227
Living with child	**1.569**	**1.074**	**2.290**	**2.078**	**1.398**	**3.088**
Married	0.900	0.541	1.498	0.664	0.304	1.450
STI sign/symptom	**2.081**	**1.472**	**2.940**	1.071	0.729	1.573
Contraception: no method vs condom / other	1.395	0.874	2.229	**2.056**	**1.319**	**3.203**
Contraception: modern vs condom / other	0.777	0.474	1.273	1.321	0.827	2.111

	Bloemfontein			Rustenburg		
Factor	**Odds Ratio**	**95% CI**		**Odds Ratio**	**95% CI**	
More than 1 partner	1.250	0.594	2.632	1.425	0.863	2.354
1 or more new partner	1.168	0.853	1.598	1.136	0.724	1.783
More than 1 different partner	0.809	0.564	1.161	0.836	0.504	1.387
1 or more sex without condom with primary partner last month	1.279	0.917	1.786	1.165	0.776	1.748
1 or more sex without condom with any other partner last month	1.067	0.622	1.831	1.224	0.444	3.379
Anal sex with primary partner	0.828	0.407	1.686	0.974	0.318	2.984
Anal sex with any other partner	0.660	0.183	2.383	1.333	0.153	11.577

Significant factors are in **bold-face.**

HIV incidence

Nine seroconversions were observed during the prospective study in Bloemfontein, an overall incidence of 5.5 per 100 PY (95% CI 2.5-10.4; Table 4). The incidence was 5.7 per 100 PY (95% CI 2.3-11.7) in women 18-24 years, 4.2 per 100 PY (95% CI 0.1-23.5) among women 25-29 years, and 5.8 per 100 PY (95% CI 0.2-32.2) in the sparse 30-35 years age stratum.

Two seroconversions were detected in Rustenburg for an overall incidence of 3.0 per 100 PY (95% CI 0.4-10.8; Table 4). Both seroconversions were in the 18-24 year age group, and the incidence in that younger age range was 4.0 per 100 PY (95% CI 0.5-14.5).

Factors associated with incident HIV

With nine new infections in the Bloemfontein cohort, we performed univariate proportional hazards regression with the same factors included in the analysis of risk factors for prevalent infection (above). Given the small number of events, no factor was significantly associated with incident infection. The strongest association with incident HIV was found for one or more unprotected coital acts with a non-primary partner in the last month (hazard ratio = 6.7; p=0.07).

Pregnancy

The prevalence of pregnancy at the time of cross-sectional screening was 6.5% (95% CI 5.0-7.9) in Bloemfontein and 8.6% (95% CI 6.2-10.9) in Rustenburg. (Further analysis of the Rustenburg pregnancy results will appear elsewhere.) In Bloemfontein, the pregnancy prevalence ranged from 11.0% (95% CI 8.5-14.0) in women not using a method to 3.5% (95% CI 1.3-7.5) in condom users and 2.1% (95% CI 0.9-3.9) in users of effective methods (hormonal and IUDs). The Bloemfontein pregnancy incidence was 16.4 per 100 PY (95% CI 10.8-23.9), and ranged from 27.6 per 100 PY (95% CI 17.1-42.2) in women not using a method at baseline to 12.4 per 100 (95% CI 2.6-36.4) in condom users and 4.7 per 100 (95% CI 1.0-13.7) in users of effective methods (hormonal or IUD).

Table 4. HIV Incidence among Women in the Prospective Study at the Bloemfontein and Rustenburg Sites

	Bloemfontein	Rustenburg
N with HIV test result during follow-up	391	194
Confirmed HIV seroconversions	9	2
Person-years of follow-up	164.4	67.1
Incidence rate[1] (95% CI[2])	5.5 (2.5-10.4)	3.0 (0.4-10.8)

[1]Per 100 person-years
[2]Exact confidence interval

DISCUSSION

HIV prevalence in Bloemfontein's Free State Province has been close to 30% in antenatal surveillance for several years. Prevalence was lower in this study, at 21.2%, yet we detected a high incidence rate of 5.5 per 100 PY overall. We observed similar HIV incidence across the age spectrum in Bloemfontein, contrary to national estimates (8). The HIV prevalence among Rustenburg women in the cross-sectional screening was 23.5%, again lower than recent antenatal surveillance data from the area. The prospective incidence rate in the Rustenburg cohort was 3.0 per 100 PY, and higher among younger women (4.0 per 100 PY in 18-24 year olds).

Ten of the eleven incident HIV infections at the two sites clearly occurred after enrollment in the prospective phase. But one seroconversion in Bloemfontein was more problematic; rapid tests were negative at cross-sectional testing and no virus was detected in that stored specimen by PCR, but Western blot results were positive for the stored baseline specimen and were persistently blot-positive until seroconversion at the three-month visit. After excluding that Bloemfontein participant, the re-calculated Bloemfontein incidence rate was 4.9 per 100 PY (2.1-9.6).

National household survey data in South Africa show that HIV prevalence has stabilized, and suggest that the incidence estimated from nationally representative surveys has declined in younger age groups (8). But the overall estimated incidence remained 1.3 per 100 uninfected persons of reproductive age from 2005-2008 (8). Others have described a pattern of stable prevalence in South African antenatal surveillance and population-based surveys coupled with substantial incidence rates in defined cohorts (9). In the relatively few studies that have directly measured HIV incidence, it has been distressingly high in both urban and rural sites, despite multiple follow-up contacts, ongoing risk-reduction counseling, and condom promotion and provision. Between 2001 and 2004, a representative cohort in one part of rural Limpopo Province had an HIV incidence of 4.9 per 100 person-years among women, despite self-reported increases in condom use during that period (10). In the HPTN 055 cohort study, the two South African sites had the highest HIV incidence rates in 2003-04 (Durban 5.3 per 100 PY; Hlabisa 6.2 per 100 PY) (11). In 2004-07, the HIV incidence rate was 6.4 per 100 PY and 6.5 per 100 PY in an urban and a rural cohort in KwaZulu-Natal Province, respectively (9). Two recent randomized trials of ineffective prevention products have also reported substantial HIV incidence at their South African sites (12-13). Finally, a cohort study conducted 2007-09 at two

sites in Northwest Province and Western Cape Province for capacity-building purposes reported 12-month incidence rates of 6.0 per 100 PY and 4.5/100 PY, similar to the rates reported here (14).

Older participants had a higher risk of prevalent HIV infection in our study than younger, reflecting their longer potential for exposure to HIV. Lesser education was also associated with baseline HIV infection, as has been found in other South African studies (10, 15). Other factors were not consistently associated with baseline infection across the sites, which may be partly due to misclassification error resulting from self-report. In fact, the striking differences in the self-reported risk behaviors between these two cohorts suggest that some self-reporting bias may have occurred. Bloemfontein participants reported high rates of multiple partnering (about two-thirds of the women), compared with national data on South African women aged 15-49 (3.7% in 2008) and data on men and women in the Free State in the same 2008 survey (14.6% in reference 16). In contrast, reports of multiple partnering at the Rustenburg site were close to national averages (12-14%) and similar to a prior representative household survey in Rustenburg (17).

One weakness of our analysis is that, partly due to the relatively short follow-up period, there were too few seroconversion events to do multivariable proportional hazards regression, and our univariate regression results for incident HIV infections were necessarily inconclusive. A limitation of the analysis of risk factors for prevalent HIV infections is that the behaviors reported at screening may not have reflected behaviors present at the time of infection. Lack of STI diagnostic testing precluded evaluating associations with other infections.

Perhaps the greatest study weakness relates to the validity of self-reported sexual behaviors, both for purposes of determining eligibility as well as for assessing risk during follow-up. The inaccuracies in self-reported sexual behavior during research interviews have been amply documented and are not unique to these studies. Although we attempted to keep the eligibility factors secret from participants and outreach staff, we do not know if volunteers (both eligible and ineligible) guessed those factors and shared them with other women in the community. There may have been an awareness among women at the Bloemfontein site in particular to over-report risky behaviors to gain entry to the study: the eligibility rate was very high there, and risk behaviors far exceeded national estimates. Alternatively, recruiters at the Bloemfontein, but not the Rustenburg, site may have simply tapped into a sub-group of women with particularly

risky behavior. Although we demonstrated substantial incidence rates at both sites, we do not know how well the cohorts represent all women in the age range who are truly at higher risk of HIV infection. During baseline and follow-up interviews with participants, we did not observe strong associations between those same behavioral factors and HIV infection.

Study strengths include the longitudinal design with high retention rates. Classification of the HIV outcome was highly accurate, using multiple rapid tests confirmed by later PCR testing. Testing of stored baseline specimens allowed us to pinpoint seroconversion and rule out acute pre-study infections. Finally, direct prospective measurement of HIV infection circumvented the lingering issues with the accuracy of cross-sectional incidence estimation.

The high HIV prevalences and incidence rates in these smaller South African cities should spur vigorous HIV prevention efforts. The need for HIV care and treatment in these areas is clear, yet during the study Free State Province experienced stock-outs of HIV antiretroviral drugs (18). Our results also highlight the success of the collaborative capacity-building SIDI projects: these sites are highly suitable and ready for HIV prevention research and programming. Both sites added staff and were provided broad research training, and these new skills were immediately applied in a rigorous clinical research study. Dealing with local ethics committees and community stakeholders, recruiting and enrolling potentially higher-risk women, administering informed consent, bringing participants back for regular study visits, giving ongoing risk-reduction and HIV counseling, and making referrals for continued health care, all within the context of Good Clinical Practice, mimicked future prevention trials. At the conclusion of its study, JOSHA in Bloemfontein was selected to participate in the Fem-PrEP randomized trial of oral HIV prophylaxis. The Aurum Institute in Rustenburg is currently conducting a clinical trial and other behavioral HIV studies. The national research capacity has been boosted and diffused to new sites outside the larger cities, which can only be a boon for future HIV and other clinical research in South Africa.

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4-6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5, 8
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-7
Bias	9	Describe any efforts to address potential sources of bias	5, 10
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7-8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7-8
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) If applicable, explain how loss to follow-up was addressed	NA
		(*e*) Describe any sensitivity analyses	NA

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	8
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8-10
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	Report numbers of outcome events or summary measures over time	11, 14
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	12-13
		(*b*) Report category boundaries when continuous variables were categorized	12
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11, 14
Discussion			
Key results	18	Summarise key results with reference to study objectives	15-16
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	16-17
Generalisability	21	Discuss the generalisability (external validity) of the study results	15-16
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	18

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

HIV incidence and prevalence among cohorts of women with higher-risk behavior in Bloemfontein and Rustenburg, South Africa: a prospective study

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HIV incidence and prevalence among cohorts of women with higher-risk behavior in Bloemfontein and Rustenburg, South Africa: a prospective study

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Running head: HIV incidence in 2 South African ~~cities~~cohorts

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ARTICLE SUMMARY

Article Focus

- Primary objective: measure HIV incidence and prevalence among women in two prospective cohorts selected for higher-risk behaviors~~of HIV-negative women~~
- Secondary objective 1: measure pregnancy rates
- Secondary objective 2: measure retention rates

Key Messages

- The project aimed to measure the HIV burden among women selected to be at higher risk in two smaller South African cities, and to prepare new clinical research groups there for future HIV prevention studies.
- HIV seroprevalence was 21.2% in Bloemfontein and 23.5% in Rustenburg.
- HIV incidence rates were 5.5 per 100 person-years (PY) in Bloemfontein and 3.0 per 100 PY in Rustenburg.

Study Strengths

- Longitudinal design with high retention rates.
- Highly accurate classification of the HIV outcome, using multiple rapid tests monthly, confirmed by later PCR testing.
- Testing of stored baseline specimens pinpointed seroconversion and ruled out acute pre-study infections.
- Direct prospective measurement of HIV infection circumvented the lingering issues with the accuracy of cross-sectional incidence estimation.

Study Limitations

- Unknown validity of self-reported sexual behaviors, both for purposes of determining eligibility and cohort definition as well as for assessing risk during follow-up.
- Relatively small study size resulted in too few seroconversion events to do multivariable Cox regression analysis, and inconclusive univariable regression results.
- The targeting of women at higher risk for HIV infection means that the prevalence and incidence figures are necessarily un-representative of women in these two cities.
- The lack of STI diagnostic testing precluded evaluating associations between HIV and other infections.

ABSTRACT

Objectives: The primary objective was to measure HIV incidence in two prospective cohorts of HIV-negative women. Secondary objectives included measuring pregnancy rates and participant retention rates.

Design: Cross-sectional HIV screening of women selected for higher-risk behaviors, with a subsequent ~~followed by a~~ prospective study of uninfected~~following initially HIV-negative~~ women followed monthly for up to 6 month~~sly visits~~.

Setting: ~~Primary health care c~~Clinics established for research purposes in Bloemfontein and Rustenburg, South Africa.

Participants: We enrolled women 18-35 years old and presumed at higher risk of sexual acquisition of HIV as indicated by self-reported sexual behavior or recent sexually transmitted infection symptoms. In Bloemfontein, ~~cross-sectional screening enrolled~~ 1364 women were screened; 1154 were eligible for HIV testing; 1145 agreed to be tested. The prospective study enrolled 401 HIV-negative women ~~from screening~~. In Rustenburg, ~~cross-sectional screening enrolled~~ 946 women were screened; 540 were eligible and underwent HIV testing; 223 HIV-negative women entered the prospective study.

Primary and secondary outcomes: Baseline prevalences of HIV infection, and HIV incidence rates in the prospective cohorts, according to a double rapid test algorithm with a third rapid test for discrepant or indeterminate results. Pregnancy prevalences and pregnancy incidence rate in Bloemfontein. Participant retention rates in the prospective cohort until study end.

Results: In Bloemfontein, 1145 women were tested~~screened~~; 391 entered follow-up; 92.3% of participants completed 6 study visits. In Rustenburg, 540 women were tested~~screened~~; 194 entered follow-up; retention up to the point of early study termination was 88.6%. Overall HIV prevalence was 21.2% (95% confidence interval [CI] 18.9-23.6) in Bloemfontein and 23.5% (95% CI 19.9-27.1) in Rustenburg. Overall HIV incidence was 5.5 per 100 person-years (PY; 95% CI 2.5-10.4) in Bloemfontein and 3.0 per 100 PY (95% CI 0.4-10.8) in Rustenburg. Cross-sectional pregnancy prevalences were 6.5% in Bloemfontein and 8.6% in Rustenburg.

Conclusions: We observed substantial HIV incidence rates in both cohorts. Vigorous prevention efforts are needed in these smaller cities.

INTRODUCTION

HIV/AIDS continues to exact a massive toll on the health, economics, and political infrastructure of communities around the world. In sub-Saharan Africa, the most heavily affected region, an estimated 22.4 million people were reported to be living with HIV in 2008 (1). While recent prevention trials assessing male circumcision, antiretroviral gel use and pre-exposure prophylaxis have shown significant promise (2-5), studies to corroborate these results and to evaluate new prevention methods will be required. To meet the ongoing need for research sites in countries with substantial HIV burden, FHI 360's Site Identification and Development Initiative (SIDI) undertook the development of previously research naïve sites in six countries to conduct future HIV prevention research.

The SIDI team partnered with groups in two South African cities for clinical research development: Bloemfontein in the Motheo District of Free State Province, and Rustenburg in the Bojanala District of Northwest Province. District-level HIV prevalence rates among antenatal women aged 15-49 were 27.8% and 34.9%, respectively in 2009; provincial HIV prevalence rates were 30.1% (Free State) and 30% (Northwest) (6). HIV incidence rates for both sites had not been measured.

The goal of the SIDI project was to develop HIV prevention research capacity and to determine the suitability of each site to implement future HIV prevention clinical trials. Each site conducted its own prevalence and incidence study using a similar protocol. The primary objective of each study was to measure HIV incidence in a prospective cohort of HIV-negative women at each study site. Secondary objectives included measuring pregnancy rates and participant accrual and retention rates in the cohort.

METHODS

Study Design and Recruitment

The study comprised cross-sectional screening with HIV testing, followed by a prospective cohort study with up to six monthly follow-up visits. In Bloemfontein, the study was conducted at the JOSHA Research Centre. Bloemfontein and the surrounding Mangaung municipality comprise a population of approximately 600,000 people. JOSHA ~~consists of a general practice and~~is an independent clinical research facility that~~and~~ can draw participants from affiliated~~the~~ general practices as well as from cooperating government clinics. Participants for the cross-sectional screening were recruited using methods developed based on feedback from

community engagement activities led by the Mangaung-University Community Partnership Program (MUCPP) and input from key stakeholders in the community. Recruitment methods included: 1) recruitment from HIV counseling and testing, sexually transmitted disease, and family planning clinics; 2) targeted outreach activities in the community; and 3) referrals from other clinics. Participants for the prospective study were recruited from among the HIV-negative participants in cross-sectional screening.

In Rustenburg, with a population estimated at 350,000 in the 2001 census but which has grown considerably since then, the study was conducted at The Aurum Institute's Rustenburg Research Centre located downtown. The Rustenburg economy is largely driven by the platinum mining industry, and is home to a large, diverse and growing influx of residents attracted to the area for work in the mining industry. The Aurum Institute (formerly the Aurum Institute for Health Research) is a non-profit research organization that began activities in March 2007 to prepare the Rustenburg Municipality to engage in epidemiologic studies and future clinical research trials. Participants for the cross-sectional screening were recruited from a variety of clinic-based and community settings which included an emphasis on local colleges. To facilitate recruitment and enrollment for cross-sectional screening, the protocol was amended twice late in the study to add respondent driven sampling (RDS) for~~as a~~ recruitment (not inference) purposes~~method~~ (7), and to liberalize the sexual behavior eligibility criterion. Both men and women were recruited for cross-sectional screening in Rustenburg, but only HIV-negative women were invited to participate in the prospective study as there was a separate male cohort study there.

Study Procedures

Following the informed consent process and assignment of a participant number, volunteers were considered enrolled. Enrolled participants were screened for eligibility for cross-sectional testing using a demographic and HIV risk factor questionnaire. Recruitment and screening staff at both sites were blind to the specific behavioral eligibility criteria to avoid the spread of information through the community, and to prevent volunteers from altering self-reported behaviors to gain access to the study. All participants were asked the full questionnaire regardless of their initial answers.

Only eligible women were tested. Eligibility for HIV and pregnancy testing was determined by age (18-35 at the time of screening) and presumed higher risk of sexual acquisition of HIV as

defined by at least one of the following self-reported criteria in the past three months: 1) treatment for, diagnosis of, or signs/symptoms of a sexually transmitted infection (STI); 2) vaginal or anal intercourse with more than one partner (unprotected intercourse in Rustenburg only); 3) vaginal or anal intercourse with a new sexual partner (unprotected intercourse in Rustenburg only); or 4) sex with an HIV-infected partner who is not using antiretroviral therapy (ART). Near the end of the Rustenburg study, we revised these criteria to remove the requirements for multiple partners and unprotected sexual acts for women, as we suspected that we were screening out many higher-risk women, and that condom use reporting was not accurate. Participants deemed eligible underwent HIV testing within the context of HIV pre- and post-test counseling in accordance with national guidelines. Women also had urine pregnancy tests. Results of both tests were provided to participants during their visits.

Women were eligible to enter the prospective cohort study if they met the cross-sectional age and behavioral criteria, were HIV- and pregnancy-negative, and were not planning to relocate from the area in the near future. The target prospective study size was 400 women at each site, followed monthly for up to 6 months. At each monthly visit, participants provided updated medical and behavioral information, underwent HIV and pregnancy testing, received syndromic management for STIs (as needed), were counseled on HIV risk reduction practices, and received free condoms.

HIV testing was performed on finger prick blood samples using a double rapid test algorithm with the Abbott Determine HIV – 1/2™ and Trinity Biotech Uni-Gold™ run in parallel. Two positive results were deemed positive and two negative results were deemed negative, which exceeded national HIV testing guidelines. Discrepant or indeterminate samples were tested with a third rapid test, SD BIOLINE HIV – 1/2 3.0, as a tie-breaker. Pregnancy testing was performed on urine samples using the Quidel QuickVue hCG-Combo test. All tests were run according to manufacturers' specifications.

Participants who tested HIV-positive at screening or during the cohort study were referred for care in accordance with local guidelines. In addition, those participants who seroconverted during the prospective phase were intensely counseled about secondary HIV prevention in the acute infection period, and in Rustenburg were invited to join a seroconvertor cohort with continued close monitoring. Participants who were found to be pregnant were referred for

antenatal care. Participants who became pregnant during follow-up continued to make study visits.

Ethical Considerations

The protocols, informed consent forms, participant education and recruitment materials, ~~and~~ case report forms and compensation amount for visits (50 rand) were reviewed and approved by the FHI ethics committee and the local ethics committees, the University of Free State ethics committee for Bloemfontein and the University of KwaZulu Natal Biomedical Research Ethics Committee for Rustenburg.

We obtained written informed consent from each participant prior to enrollment and data collection. Written consent was also obtained for long-term specimen storage and possible future testing, although consent for specimen storage was not required for study participation. Informed consent forms were available in English and were translated into local languages, Sesotho (Bloemfontein) and Sestwana and IsiXhosa (Rustenburg) to enhance comprehension. Volunteers had their choice of language for the informed consent process, which was witnessed and verified by a non-study staff person for illiterate or low-literate participants.

Statistical Analysis

All eligible participants were included in the data analysis. For each site, we summarized participants' socio-demographic characteristics, self-reported STI symptoms and HIV risk behaviors at baseline for cross-sectional screening participants, and those women who entered the prospective study. We calculated baseline prevalences of HIV infection and pregnancy among women, along with 95% confidence intervals (CI). We used univariate and multivariable logistic regression analysis to evaluate the association between risk factors and prevalent HIV infection.

The incidence rate was calculated as the number of post-enrollment HIV seroconversions divided by the person-years (PY) of observation accumulated by the cohort. We calculated 95% CI using exact methods under the assumption that the number of HIV infections follows a Poisson distribution. We used proportional hazards regression to evaluate the associations between incident HIV infection and possible HIV risk factors including time-dependent participant characteristics, and time-varying risk behaviors and self-reported STI symptoms.

Due to the small number of HIV seroconversions, we did univariate proportional hazards regression only. Data analyses were performed using SAS version 9.2 (Cary, North Carolina).

RESULTS

Eligible populations

In Bloemfontein, the cross-sectional screening enrolled 1364 women between February and October 2009. Of the 1364 enrolled women, 1154 were determined to be eligible for testing, and 1145 (84% of enrollees) chose to be tested in cross-sectional screening. Virtually all of the women who were screened out from cross-sectional testing failed to satisfy the behavioral or STI risk criteria. The prospective study enrolled 401 HIV-negative women volunteers from screening. Of those 401 women, 399 were determined to be eligible, and 391 entered follow-up and were tested. ~~Retention in the prospective study was excellent, with~~ 92.3% of participants complet~~ed~~ing 6 study visits~~, with~~ for a median 5.6 person-months of observation and a total 164.4 person-years.

In Rustenburg, cross-sectional screening enrolled 946 women and 968 men between November 2008 and December 2009. Data on the male participants are not presented here. Of the 946 enrolled women, 540 (57%) were eligible and underwent testing in cross-sectional screening; virtually all~~most~~ of the women who were screened out from cross-sectional testing did not satisfy the behavioral or STI risk criteria. The prospective study enrolled 223 HIV-negative women volunteers, of whom 194 yielded HIV test data. In Rustenburg, many women screened out from the prospective study due to plans to re-locate. Completion of follow-up in the prospective study was hindered when it was ended prematurely due to funding shortfalls, but retention in follow-up up to the point of study termination was 88.6% with a median 4.1 person-months of observation and a total 67.1 person-years.

Participant characteristics

Large percentages of participants in cross-sectional screening at both sites were ≤21 years, had a high school level education, were not employed, were unmarried, and lived with family members (Table 1). About one-third of the women were using an effective contraceptive at each site (hormonal method or IUD: 38.3% in Bloemfontein and 32.9% in Rustenburg; Table 1). STI testing was not done; self-reported STI signs/symptoms at baseline ranged from 2-15% prevalence in Bloemfontein, and from 22-37% in Rustenburg.

At baseline, women in the Bloemfontein study self-reported more risk behaviors than women in Rustenburg (Table 1). Two-thirds of women in Bloemfontein reported vaginal intercourse with two or more partners in the month preceding cross-sectional screening; the corresponding percentage in Rustenburg was 14%. Over half of the women in Bloemfontein reported having vaginal intercourse with their primary partners without a condom in the previous week, whereas about a quarter of women in Rustenburg reported unprotected intercourse with primary partners in the previous week. Women in Bloemfontein were also more likely to report unprotected intercourse with a non-primary partner.

Table 1. Participant Features (N & %) of Eligible Women at Inception of Cross-sectional Screening and Prospective Study in Bloemfontein and Rustenburg Sites

	Bloemfontein		Rustenburg	
	Cross-sectional N = 1154	**Prospective N = 399**	**Cross-sectional N = 540**	**Prospective N = 223**
Age				
<21	520 (45.1)	206 (51.6)	239 (44.2)	115 (51.6)
22-24	259 (22.4)	92 (23.1)	130 (24.1)	53 (23.8)
25-31	284 (24.6)	76 (19.1)	138 (25.6)	46 (20.6)
>31	91 (7.9)	25 (6.3)	33 (6.1)	9 (4.0)
Education				
None - grade 5	32 (2.8)	7 (1.8)	4 (0.8)	0 (0.0)
Grade 6-9	181 (15.7)	49 (12.3)	27 (5.0)	10 (4.5)
Grade 10-12	860 (74.5)	333 (83.5)	454 (84.1)	188 (84.3)
Univ/college/grad	81 (7.0)	10 (2.5)	55 (10.2)	25 (11.2)
Employment				
No, unemployed	1000 (86.7)	352 (88.2)	463 (85.7)	196 (87.9)
Yes, part-time	78 (6.8)	25 (6.3)	31 (5.7)	11 (4.9)
Yes, full-time	76 (6.6)	22 (5.5)	46 (8.5)	16 (7.2)
Whom you live with				
Alone	45 (3.9)	12 (3.0)	25 (4.6)	8 (3.6)
Spouse	52 (4.5)	15 (3.8)	15 (2.8)	8 (3.6)
Family/relatives	859 (74.4)	315 (78.9)	375 (69.4)	160 (71.8)
Roommate/friend	103 (8.9)	34 (8.5)	28 (5.2)	12 (5.4)
Partner	95 (8.2)	23 (5.8)	97 (18.0)	35 (15.7)

	Bloemfontein		Rustenburg	
	Cross-sectional N = 1154	Prospective N = 399	Cross-sectional N = 540	Prospective N = 223
Married				
No	1056 (91.5)	369 (92.5)	507 (93.9)	211 (94.6)
Yes	98 (8.5)	30 (7.5)	33 (6.1)	12 (5.4)
Contraceptive use				
None	540 (46.9)	182 (45.6)	157 (29.1)	58 (26.0)
Oral	55 (4.8)	20 (5.0)	24 (4.4)	19 (8.5)
Injectable	384 (33.3)	138 (34.6)	132 (24.4)	65 (29.2)
IUD	2 (0.2)	0 (0.0)	1 (0.2)	1 (0.5)
Condoms	148 (12.9)	52 (13.0)	201 (37.2)	80 (35.9)
Other	23 (2.0)	7 (1.8)	1 (0.2)	0 (0.0)
Missing	2 (0.2)	0 (0.0)	24 (4.4)	0 (0.0)
STI signs/symptoms				
Vaginal discharge	155 (13.4)	52 (13.0)	154 (28.5)	59 (26.5)
Painful urination	103 (8.9)	31 (7.8)	153 (28.3)	57 (25.6)
Lower abd. pain	115 (10.0)	39 (9.8)	202 (37.4)	82 (36.8)
Vaginal itching	170 (14.7)	60 (15.0)	155 (28.7)	63 (28.5)
Dyspareunia	104 (9.0)	35 (8.8)	121 (22.4)	50 (22.4)
Vaginal sore	27 (2.3)	12 (3.0)	142 (26.3)	66 (29.6)
No. sex partners in last month				
0	53 (4.6)	18 (4.5)	85 (15.7)	40 (17.9)
1	362 (31.4)	120 (30.1)	400 (74.1)	155 (69.5)
2+	739 (64.0)	261 (65.4)	55 (10.2)	28 (12.6)
Vaginal sex w/o condom past week w primary partner	638 (55.3)	202 (50.6)	190 (35.2)	78 (35.0)
Vaginal sex w/o condom past week w other partner	150 (13.0)	50 (12.5)	8 (1.5)	3 (1.3)

The baseline features of women who entered the prospective studies were broadly similar to those of the source screening populations at both sites (Table 1), and there was little indication of selection for a particular subgroup into the prospective cohort.

HIV prevalence

In Bloemfontein cross-sectional screening, the HIV prevalence ~~in cross-sectional screening~~ was 21.2% (95% confidence interval [CI] 18.9-23.6) overall: 13.4% in those 18-24 years; 38.1% in those 25-29 years; and 37.1% in women aged 30-35 years (Table 2). In Rustenburg, the overall HIV prevalence at screening was 23.5% (95% CI 19.9-27.1) among women, ranging from 18.7% in those age 18-24 to 31.8% in those age 25-29 to 37.7% in those age 30-35 (Table 2).

Table 2. HIV Prevalence among Women in Cross-sectional Screening by Age Group and Research Site

	Bloemfontein	Rustenburg
Age 18-24		
Eligible Population[1], N	776	369
HIV positive, N	104	69
Prevalence (95% CI[2])	13.4% (11.0-15.8)	18.7% (14.7-22.7)
Age 25-29		
Eligible Population[1], N	226	110
HIV positive, N	86	35
Prevalence (95% CI[2])	38.1% (31.7-44.4)	31.8% (23.1-40.5)
Age 30+		
Eligible Population[1], N	143	61
HIV positive, N	53	23
Prevalence (95% CI[2])	37.1% (29.1-45.0)	37.7% (25.5-49.9)
TOTAL		
Eligible Population[1], N	1145	540
HIV positive, N	243	127
Prevalence (95% CI[2])	21.2% (18.9- 23.6)	23.5% (19.9- 27.1)

[1] Eligible and had HIV test result
[2] Approximate confidence interval

Factors associated with prevalent HIV

Three baseline factors were significantly associated with prevalent HIV infection at both study sites (Table 3): age 30 or older compared with 18-24; less versus more educational attainment; and living with a child versus not living with child. Self-reported signs or symptoms of STI were

associated with HIV in Bloemfontein only, and non-use of contraception versus use of condoms was associated with HIV in Rustenburg only. Sexual behavior factors were not significantly associated with prevalent HIV infection.

Table 3. Multivariable Logistic Regression Analysis of Factors Associated with Prevalent HIV among Women Screened inat the Bloemfontein and Rustenburg, South Africa Sites

	Bloemfontein			Rustenburg		
Factor	**Odds Ratio**	**95% CI**		**Odds Ratio**	**95% CI**	
Age 18-24 vs 30+	**0.389**	**0.243**	**0.623**	**0.485**	**0.284**	**0.826**
Age 24-29 vs 30+	1.222	0.766	1.950	0.844	0.502	1.419
Education: Less than High school	**2.240**	**1.552**	**3.233**	**1.979**	**1.099**	**3.563**
Employed: yes	0.913	0.587	1.419	0.790	0.509	1.227
Living with child	**1.569**	**1.074**	**2.290**	**2.078**	**1.398**	**3.088**
Married	0.900	0.541	1.498	0.664	0.304	1.450
STI sign/symptom	**2.081**	**1.472**	**2.940**	1.071	0.729	1.573
Contraception: no method vs condom / other	1.395	0.874	2.229	**2.056**	**1.319**	**3.203**

	Bloemfontein			Rustenburg		
Factor	**Odds Ratio**	**95% CI**		**Odds Ratio**	**95% CI**	
Contraception: effective~~modern~~ vs condom / other	0.777	0.474	1.273	1.321	0.827	2.111
More than 1 partner	1.250	0.594	2.632	1.425	0.863	2.354
1 or more new partner	1.168	0.853	1.598	1.136	0.724	1.783
More than 1 different partner	0.809	0.564	1.161	0.836	0.504	1.387
1 or more sex without condom with primary partner last month	1.279	0.917	1.786	1.165	0.776	1.748
1 or more sex without condom with any other partner last month	1.067	0.622	1.831	1.224	0.444	3.379
Anal sex with primary partner	0.828	0.407	1.686	0.974	0.318	2.984
Anal sex with any other partner	0.660	0.183	2.383	1.333	0.153	11.577

Significant factors are in **bold-face.**

HIV incidence

Nine seroconversions were observed during the prospective study in the Bloemfontein cohort, an overall incidence of 5.5 per 100 PY (95% CI 2.5-10.4; Table 4). The incidence was 5.7 per

100 PY (95% CI 2.3-11.7) in women 18-24 years, 4.2 per 100 PY (95% CI 0.1-23.5) among women 25-29 years, and 5.8 per 100 PY (95% CI 0.2-32.2) in the sparse 30-35 years age stratum.

Two seroconversions were detected in the Rustenburg cohort for an overall incidence of 3.0 per 100 PY (95% CI 0.4-10.8; Table 4). Both seroconversions were in the 18-24 year age group, and the incidence in that younger age range was 4.0 per 100 PY (95% CI 0.5-14.5).

Table 4. HIV Incidence among Women in the Prospective Study at the Bloemfontein and Rustenburg Sites

	Bloemfontein	Rustenburg
N with HIV test result during follow-up	391	194
Confirmed HIV seroconversions	9	2
Person-years of follow-up	164.4	67.1
Incidence rate[1] (95% CI[2])	5.5 (2.5-10.4)	3.0 (0.4-10.8)

[1]Per 100 person-years
[2]Exact confidence interval

Ten of the eleven incident HIV infections at the two sites clearly occurred after enrollment in the prospective phase. One seroconversion in Bloemfontein was more problematic; rapid tests were negative at cross-sectional testing and no virus was detected in that stored specimen by PCR, but Western blot results were positive for the stored baseline specimen and were persistently blot-positive until seroconversion at the three-month visit. After excluding that Bloemfontein participant, the re-calculated Bloemfontein incidence rate was 4.9 per 100 PY (95% CI 2.1-9.6).

Factors associated with incident HIV

With nine new infections in the Bloemfontein cohort, we performed univariate proportional hazards regression with the same factors included in the analysis of risk factors for prevalent infection (above). ~~Given the small number of events, n~~No factor was significantly associated

with incident infection. The strongest association with incident HIV was found for one or more unprotected coital acts with a non-primary partner in the last month (hazard ratio = 6.7; p=0.07).

Pregnancy

The prevalence of pregnancy at the time of cross-sectional screening was 6.5% (95% CI 5.0-7.9) in Bloemfontein and 8.6% (95% CI 6.2-10.9) in Rustenburg. (Further analysis of the Rustenburg pregnancy results will appear elsewhere.) In Bloemfontein, the pregnancy prevalence in screened women ranged from 11.0% (95% CI 8.5-14.0) in thosewomen not using a method to 3.5% (95% CI 1.3-7.5) in condom users and 2.1% (95% CI 0.9-3.9) in users of effective methods (hormonal and IUDs). The Bloemfontein pregnancy incidence was 16.4 per 100 PY (95% CI 10.8-23.9), and ranged from 27.6 per 100 PY (95% CI 17.1-42.2) in women not using a method at baseline to 12.4 per 100 (95% CI 2.6-36.4) in condom users and 4.7 per 100 (95% CI 1.0-13.7) in users of effective methods (hormonal or IUD).

DISCUSSION

HIV prevalence in Bloemfontein's Free State Province has been close to 30% in antenatal surveillance for several years. Prevalence was lower in this study, at 21.2%, yet we detected a high incidence rate of 5.5 per 100 PY in the overall cohortoverall. We observed similar HIV incidence across the age spectrum in the Bloemfontein cohort, contrary to national estimates (8). The HIV prevalence among Rustenburg women in the cross-sectional screening was 23.5%, again lower than recent antenatal surveillance data from the area, which could be explained by the young average age of the cohort screened. The prospective incidence rate in the Rustenburg cohort was 3.0 per 100 PY, and higher among younger women (4.0 per 100 PY in 18-24 year olds).

~~Ten of the eleven incident HIV infections at the two sites clearly occurred after enrollment in the prospective phase. But one seroconversion in Bloemfontein was more problematic; rapid tests were negative at cross-sectional testing and no virus was detected in that stored specimen by PCR, but Western blot results were positive for the stored baseline specimen and were persistently blot-positive until seroconversion at the three-month visit. After excluding that Bloemfontein participant, the re-calculated Bloemfontein incidence rate was 4.9 per 100 PY (2.1-9.6).~~

National household survey data in South Africa show that HIV prevalence has stabilized, and suggest that the incidence estimated from nationally representative surveys has declined in younger age groups (8). But the overall estimated incidence remained 1.3 per 100 uninfected persons of reproductive age from 2005-2008 (8). Others have described a pattern of stable prevalence in South African antenatal surveillance and population-based surveys coupled with substantial incidence rates in defined cohorts (9). In the relatively few studies that have directly measured HIV incidence, it has been distressingly high in both urban and rural sites, despite multiple follow-up contacts, ongoing risk-reduction counseling, and condom promotion and provision. Between 2001 and 2004, a representative cohort in one part of rural Limpopo Province had an HIV incidence of 4.9 per 100 person-years among women, despite self-reported increases in condom use during that period (10). In the HPTN 055 cohort study, the two South African sites, both located in Kwa-Zulu Natal Province, had the highest HIV incidence rates in 2003-04 (Durban 5.3 per 100 PY; Hlabisa 6.2 per 100 PY) (11). In 2004-07, the HIV incidence rate was 6.4 per 100 PY and 6.5 per 100 PY in an urban and a rural cohort in KwaZulu-Natal ~~Province~~, respectively (9). Threewo recent randomized trials of ineffective prevention products have also reported substantial HIV incidence at their South African sites (12-~~13~~14). Finally, a cohort study conducted 2007-09 at two sites in Northwest Province and Western Cape Province for capacity-building purposes reported 12-month incidence rates of 6.0 per 100 PY and 4.5/100 PY, similar to the rates reported here (~~14~~15).

Older participants had a higher risk of prevalent HIV infection in our study than younger, reflecting their longer potential for exposure to HIV. Lesser education was also associated with baseline HIV infection, as has been found in other South African studies (10, ~~15~~16). Other factors were not consistently associated with baseline infection across the sites, which may stem from unmeasured sexual network factors, and~~be partly due to~~ misclassification error resulting from self-report. In fact, the striking differences in the self-reported risk behaviors between these two cohorts suggest that some self-reporting bias may have occurred. Bloemfontein participants reported high rates of multiple partnering (about two-thirds of the women), compared with national data on South African women aged 15-49 (3.7% in 2008) and data on men and women in the Free State in the same 2008 survey (14.6% in reference ~~16~~17). In contrast, reports of multiple partnering at the Rustenburg site were close to national averages (12-14%) and similar to a prior representative household survey in Rustenburg (~~17~~18).

One weakness of our analysis is that, partly due to the relatively short follow-up period, there were too few seroconversion events to do multivariable proportional hazards regression, and our univariate regression results for incident HIV infections were necessarily inconclusive. A limitation of the analysis of risk factors for prevalent HIV infections is that the behaviors reported at screening may not have reflected behaviors present at the time of infection. Lack of STI diagnostic testing precluded evaluating associations with other infections.

Perhaps the greatest study weakness relates to the validity of self-reported sexual behaviors, both for purposes of determining eligibility as well as for assessing risk during follow-up. The inaccuracies in self-reported sexual behavior during research interviews have been amply documented and are not unique to these studies. Although we attempted to keep the eligibility factors secret from participants and outreach staff, we do not know if volunteers (both eligible and ineligible) guessed those factors and shared them with other women in the community. There may have been an awareness among women at the Bloemfontein site in particular to over-report risky behaviors to gain entry to the study: the eligibility rate was very high there, and risk behaviors far exceeded national estimates. Alternatively, recruiters at the Bloemfontein, but not the Rustenburg, site may have simply tapped into a sub-group of women with particularly risky behavior. ~~Although we demonstrated substantial incidence rates at both sites, we do not know how well the cohorts represent all women in the age range who are truly at higher risk of HIV infection.~~ During baseline and follow-up interviews with participants, however, we did not observe strong associations between those same behavioral factors and HIV infection.

Our study cohorts cannot be considered representative of young women in the two cities. Our behavioral eligibility criteria selected for a group of women with higher than average risk behaviors. The Rustenburg site offered the opportunity to demonstrate the impact of these selection factors: a representative household survey had been done there in 2008, one year before our study commenced. Our study cohort was younger, better educated, less likely to be employed, and less likely to be married or live with a steady partner than all Rustenburg community residents. Most critically, only 3.4% of women in the household survey reported multiple sexual partners in the past three months, compared with over 10% of women screened in our study who reported multiple partners in the past one month. So we make no claims for representativeness: our project deliberately set out to identify and follow a cohort with higher than average risk behaviors, in order to demonstrate the suitability of the sites for future prevention research.

Countervailing sStudy strengths include the longitudinal design with high retention rates. Classification of the HIV outcome was highly accurate, using multiple monthly rapid tests confirmed by later PCR testing. Testing of stored baseline specimens allowed us to pinpoint seroconversion and rule out acute pre-study infections. Finally, direct prospective measurement of HIV infection circumvented the lingering issues with the accuracy of cross-sectional incidence estimation.

The high HIV prevalences and incidence rates in these cohorts in twothese smaller South African cities should spur vigorous HIV prevention efforts. The need for HIV care and treatment in these areas is clear, and while care is available,yet during the study Free State Province experienced stock-outs of HIV antiretroviral drugs during the study (1819). Our results also highlight the success of the collaborative capacity-building SIDI projects: these sites are highly suitable and ready for HIV prevention research and programming. Both sites added staff and were provided broad research training, and these new skills were immediately applied in a rigorous clinical research study. Dealing with local ethics committees and community stakeholders, recruiting and enrolling potentially higher-risk women, administering informed consent, bringing participants back for regular study visits, giving ongoing risk-reduction and HIV counseling, and making referrals for continued health care, all within the context of Good Clinical Practice, mimicked future prevention trials. At the conclusion of its study, JOSHA in Bloemfontein was selected to implement anparticipate in the Fem-PrEP randomized trial of oral HIV prophylaxis trial. The Aurum Institute in Rustenburg is currently conducting a clinical trial of a vaginal microbicide, along with and other behavioral HIV studies. The national research capacity has been boosted and diffused to new sites outside the larger cities, which can only be a boon for future HIV and other clinical research in South Africa.

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The authors declare no competing interests.

PJF, MHL, SFoulkes, JL and CS contributed to the design of the study and its essential documents. MHL, SFoulkes, CC, IR, GV and JL collected the data. PJF, MHL, P-LChen, and SFischer contributed to the analysis and interpretation of the data. PJF, MHL, CC, and SFischer drafted the article. All authors approved the final version of the manuscript.

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4-6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5, 8
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-7
Bias	9	Describe any efforts to address potential sources of bias	5, 10
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7-8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7-8
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) If applicable, explain how loss to follow-up was addressed	NA
		(*e*) Describe any sensitivity analyses	NA

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	8
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8-10
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	Report numbers of outcome events or summary measures over time	11, 14
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	12-13
		(*b*) Report category boundaries when continuous variables were categorized	12
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11, 14
Discussion			
Key results	18	Summarise key results with reference to study objectives	15-16
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	16-17
Generalisability	21	Discuss the generalisability (external validity) of the study results	15-16
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	18

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

HIV incidence and prevalence among cohorts of women with higher-risk behavior in Bloemfontein and Rustenburg, South Africa: a prospective study

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HIV incidence and prevalence among cohorts of women with higher-risk behavior in Bloemfontein and Rustenburg, South Africa: a prospective study

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Running head: HIV incidence in 2 South African cohorts

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ARTICLE SUMMARY

Article Focus

- Primary objective: measure HIV incidence and prevalence among women in two prospective cohorts selected for higher-risk behaviors
- Secondary objective 1: measure pregnancy rates
- Secondary objective 2: measure retention rates

Key Messages

- We measured the HIV burden among women selected to be at higher risk in two smaller South African cities, while training new clinical research groups there.
- HIV seroprevalence was 21.2% in Bloemfontein and 23.5% in Rustenburg.
- HIV incidence rates were 5.5 per 100 person-years (PY) in Bloemfontein and 3.0 per 100 PY in Rustenburg.

Study Strengths

- Longitudinal design with high retention rates.
- Highly accurate classification of the HIV outcome, using multiple rapid tests monthly, confirmed by later PCR testing.
- Testing of stored baseline specimens to pinpoint seroconversion and rule out acute pre-study infections.
- Direct prospective measurement of HIV infection circumvented the lingering issues with the accuracy of cross-sectional incidence estimation.

Study Limitations

- Unknown validity of self-reported sexual behaviors.
- Relatively small study size precluded multivariable Cox regression analysis.
- Selective recruitment means that the prevalence and incidence figures are not representative of women in these two cities.
- The lack of STI diagnostic testing precluded evaluating associations between HIV and other infections.

ABSTRACT

Objectives: The primary objective was to measure HIV incidence in two prospective cohorts of HIV-negative women. Secondary objectives included measuring pregnancy rates and participant retention rates.

Design: Cross-sectional HIV screening of women selected for higher-risk behaviors, with a subsequent prospective study of uninfected women followed monthly for up to 6 months.

Setting: Clinics established for research purposes in Bloemfontein and Rustenburg, South Africa.

Participants: We enrolled women 18-35 years old and presumed at higher risk of sexual acquisition of HIV as indicated by self-reported sexual behavior or recent sexually transmitted infection symptoms. In Bloemfontein, 1364 women were screened; 1154 were eligible for HIV testing; 1145 agreed to be tested. The prospective study enrolled 401 HIV-negative women. In Rustenburg, 946 women were screened; 540 were eligible and underwent HIV testing; 223 HIV-negative women entered the prospective study.

Primary and secondary outcomes: Baseline prevalences of HIV infection, and HIV incidence rates in the prospective cohorts, according to a double rapid test algorithm with a third rapid test for discrepant or indeterminate results. Pregnancy prevalences and pregnancy incidence rate in Bloemfontein. Participant retention rates in the prospective cohort until study end.

Results: In Bloemfontein, 1145 women were tested; 391 entered follow-up; 92.3% of participants completed 6 study visits. In Rustenburg, 540 women were tested; 194 entered follow-up; retention up to the point of early study termination was 88.6%. Overall HIV prevalence was 21.2% (95% confidence interval [CI] 18.9-23.6) in Bloemfontein and 23.5% (95% CI 19.9-27.1) in Rustenburg. Overall HIV incidence was 5.5 per 100 person-years (PY; 95% CI 2.5-10.4) in Bloemfontein and 3.0 per 100 PY (95% CI 0.4-10.8) in Rustenburg. Cross-sectional pregnancy prevalences were 6.5% in Bloemfontein and 8.6% in Rustenburg.

Conclusions: We observed substantial HIV incidence rates in both cohorts. Vigorous prevention efforts are needed in these smaller cities.

INTRODUCTION

HIV/AIDS continues to exact a massive toll on the health, economics, and political infrastructure of communities around the world. In sub-Saharan Africa, the most heavily affected region, an estimated 22.4 million people were reported to be living with HIV in 2008 (1). While recent prevention trials assessing male circumcision, antiretroviral gel use and pre-exposure prophylaxis have shown significant promise (2-5), studies to corroborate these results and to evaluate new prevention methods will be required. To meet the ongoing need for research sites in countries with substantial HIV burden, FHI 360's Site Identification and Development Initiative (SIDI) undertook the development of previously research naïve sites in six countries to conduct future HIV prevention research.

The SIDI team partnered with groups in two South African cities for clinical research development: Bloemfontein in the Motheo District of Free State Province, and Rustenburg in the Bojanala District of Northwest Province. District-level HIV prevalence rates among antenatal women aged 15-49 were 27.8% and 34.9%, respectively in 2009; provincial HIV prevalence rates were 30.1% (Free State) and 30% (Northwest) (6). HIV incidence rates for both sites had not been measured.

The goal of the SIDI project was to develop HIV prevention research capacity and to determine the suitability of each site to implement future HIV prevention clinical trials. Each site conducted its own prevalence and incidence study using a similar protocol. The primary objective of each study was to measure HIV incidence in a prospective cohort of HIV-negative women at each study site. Secondary objectives included measuring pregnancy rates and participant accrual and retention rates in the cohort.

METHODS

Study Design and Recruitment

The study comprised cross-sectional screening with HIV testing, followed by a prospective cohort study with up to six monthly follow-up visits. In Bloemfontein, the study was conducted at the JOSHA Research Centre. Bloemfontein and the surrounding Mangaung municipality comprise a population of approximately 600,000 people. JOSHA is an independent clinical research facility that can draw participants from affiliated general practices as well as from cooperating government clinics. Participants for the cross-sectional screening were recruited using methods developed based on feedback from community engagement activities led by the

Mangaung-University Community Partnership Program (MUCPP) and input from key stakeholders in the community. Recruitment methods included: 1) recruitment from HIV counseling and testing, sexually transmitted disease, and family planning clinics; 2) targeted outreach activities in the community; and 3) referrals from other clinics. Participants for the prospective study were recruited from among the HIV-negative participants in cross-sectional screening.

In Rustenburg, with a population estimated at 350,000 in the 2001 census but which has grown considerably since then, the study was conducted at The Aurum Institute's Rustenburg Research Centre located downtown. The Rustenburg economy is largely driven by the platinum mining industry, and is home to a large, diverse and growing influx of residents attracted to the area for work in the mining industry. The Aurum Institute (formerly the Aurum Institute for Health Research) is a non-profit research organization that began activities in March 2007 to prepare the Rustenburg Municipality to engage in epidemiologic studies and future clinical research trials. Participants for the cross-sectional screening were recruited from a variety of clinic-based and community settings which included an emphasis on local colleges. To facilitate recruitment and enrollment for cross-sectional screening, the protocol was amended twice late in the study to add respondent driven sampling (RDS) for recruitment (not inference) purposes (7), and to liberalize the sexual behavior eligibility criterion. Both men and women were recruited for cross-sectional screening in Rustenburg, but only HIV-negative women were invited to participate in the prospective study as there was a separate male cohort study there.

Study Procedures

Following the informed consent process and assignment of a participant number, volunteers were considered enrolled. Enrolled participants were screened for eligibility for cross-sectional testing using a demographic and HIV risk factor questionnaire. Recruitment and screening staff at both sites were blind to the specific behavioral eligibility criteria to avoid the spread of information through the community, and to prevent volunteers from altering self-reported behaviors to gain access to the study. All participants were asked the full questionnaire regardless of their initial answers.

Only eligible women were tested. Eligibility for HIV and pregnancy testing was determined by age (18-35 at the time of screening) and presumed higher risk of sexual acquisition of HIV as defined by at least one of the following self-reported criteria in the past three months: 1)

treatment for, diagnosis of, or signs/symptoms of a sexually transmitted infection (STI); 2) vaginal or anal intercourse with more than one partner (unprotected intercourse in Rustenburg only); 3) vaginal or anal intercourse with a new sexual partner (unprotected intercourse in Rustenburg only); or 4) sex with an HIV-infected partner who is not using antiretroviral therapy (ART). Near the end of the Rustenburg study, we revised these criteria to remove the requirements for multiple partners and unprotected sexual acts for women, as we suspected that we were screening out many higher-risk women, and that condom use reporting was not accurate. Participants deemed eligible underwent HIV testing within the context of HIV pre- and post-test counseling in accordance with national guidelines. Women also had urine pregnancy tests. Results of both tests were provided to participants during their visits.

Women were eligible to enter the prospective cohort study if they met the cross-sectional age and behavioral criteria, were HIV- and pregnancy-negative, and were not planning to relocate from the area in the near future. The target prospective study size was the first 400 eligible women at each site, followed monthly for up to 6 months. At each monthly visit, participants provided updated medical and behavioral information, underwent HIV and pregnancy testing, received syndromic management for STIs (as needed), were counseled on HIV risk reduction practices, and received free condoms.

HIV testing was performed on finger prick blood samples using a double rapid test algorithm with the Abbott Determine HIV – 1/2™ and Trinity Biotech Uni-Gold™ run in parallel. Two positive results were deemed positive and two negative results were deemed negative, which exceeded national HIV testing guidelines. Discrepant or indeterminate samples were tested with a third rapid test, SD BIOLINE HIV – 1/2 3.0, as a tie-breaker. Pregnancy testing was performed on urine samples using the Quidel QuickVue hCG-Combo test. All tests were run according to manufacturers' specifications.

Participants who tested HIV-positive at screening or during the cohort study were referred for care in accordance with local guidelines. In addition, those participants who seroconverted during the prospective phase were intensely counseled about secondary HIV prevention in the acute infection period, and in Rustenburg were invited to join a seroconvertor cohort with continued close monitoring. Participants who were found to be pregnant were referred for antenatal care. Participants who became pregnant during follow-up continued to make study visits.

Ethical Considerations

The protocols, informed consent forms, participant education and recruitment materials, case report forms and compensation amount for visits (50 rand) were reviewed and approved by the FHI ethics committee and the local ethics committees, the University of Free State ethics committee for Bloemfontein and the University of KwaZulu Natal Biomedical Research Ethics Committee for Rustenburg.

We obtained written informed consent from each participant prior to enrollment and data collection. Written consent was also obtained for long-term specimen storage and possible future testing, although consent for specimen storage was not required for study participation. Informed consent forms were available in English and were translated into local languages, Sesotho (Bloemfontein) and Sestwana and IsiXhosa (Rustenburg) to enhance comprehension. Volunteers had their choice of language for the informed consent process, which was witnessed and verified by a non-study staff person for illiterate or low-literate participants.

Statistical Analysis

All eligible participants were included in the data analysis. For each site, we summarized participants' socio-demographic characteristics, self-reported STI symptoms and HIV risk behaviors at baseline for cross-sectional screening participants, and those women who entered the prospective study. We calculated baseline prevalences of HIV infection and pregnancy among women, along with 95% confidence intervals (CI). We used univariate and multivariable logistic regression analysis to evaluate the association between risk factors and prevalent HIV infection.

The incidence rate was calculated as the number of post-enrollment HIV seroconversions divided by the person-years (PY) of observation accumulated by the cohort. We calculated 95% CI using exact methods under the assumption that the number of HIV infections follows a Poisson distribution. We used proportional hazards regression to evaluate the associations between incident HIV infection and possible HIV risk factors including time-dependent participant characteristics, and time-varying risk behaviors and self-reported STI symptoms. Due to the small number of HIV seroconversions, we did univariate proportional hazards regression only. Data analyses were performed using SAS version 9.2 (Cary, North Carolina).

RESULTS

Eligible populations

In Bloemfontein, the cross-sectional screening enrolled 1364 women between February and October 2009. Of the 1364 enrolled women, 1154 were determined to be eligible for testing, and 1145 (84% of enrollees) chose to be tested in cross-sectional screening. Virtually all of the women who were screened out from cross-sectional testing failed to satisfy the behavioral or STI risk criteria. The prospective study enrolled 401 HIV-negative women volunteers from screening. Of those 401 women, 399 were determined to be eligible, and 391 entered follow-up and were tested. 92.3% of participants completed 6 study visits, for a median 5.6 person-months of observation and a total 164.4 person-years.

In Rustenburg, cross-sectional screening enrolled 946 women and 968 men between November 2008 and December 2009. Data on the male participants are not presented here. Of the 946 enrolled women, 540 (57%) were eligible and underwent testing in cross-sectional screening; virtually all of the women who were screened out from cross-sectional testing did not satisfy the behavioral or STI risk criteria. The prospective study enrolled 223 HIV-negative women volunteers, of whom 194 yielded HIV test data. In Rustenburg, many women screened out from the prospective study due to plans to re-locate. Completion of follow-up in the prospective study was hindered when it was ended prematurely due to funding shortfalls, but retention in follow-up up to the point of study termination was 88.6% with a median 4.1 person-months of observation and a total 67.1 person-years.

Participant characteristics

Large percentages of participants in cross-sectional screening at both sites were ≤21 years, had a high school level education, were not employed, were unmarried, and lived with family members (Table 1). About one-third of the women were using an effective contraceptive at each site (hormonal method or IUD: 38.3% in Bloemfontein and 32.9% in Rustenburg; Table 1). STI testing was not done; self-reported STI signs/symptoms at baseline ranged from 2-15% prevalence in Bloemfontein, and from 22-37% in Rustenburg.

At baseline, women in the Bloemfontein study self-reported more risk behaviors than women in Rustenburg (Table 1). Two-thirds of women in Bloemfontein reported vaginal intercourse with two or more partners in the month preceding cross-sectional screening; the corresponding percentage in Rustenburg was 14%. Over half of the women in Bloemfontein reported having

vaginal intercourse with their primary partners without a condom in the previous week, whereas about a quarter of women in Rustenburg reported unprotected intercourse with primary partners in the previous week. Women in Bloemfontein were also more likely to report unprotected intercourse with a non-primary partner.

Table 1. Participant Features (N & %) of Eligible Women at Inception of Cross-sectional Screening and Prospective Study in Bloemfontein and Rustenburg Sites

	Bloemfontein		Rustenburg	
	Cross-sectional N = 1154	**Prospective N = 399**	**Cross-sectional N = 540**	**Prospective N = 223**
Age				
<21	520 (45.1)	206 (51.6)	239 (44.2)	115 (51.6)
22-24	259 (22.4)	92 (23.1)	130 (24.1)	53 (23.8)
25-31	284 (24.6)	76 (19.1)	138 (25.6)	46 (20.6)
>31	91 (7.9)	25 (6.3)	33 (6.1)	9 (4.0)
Education				
None - grade 5	32 (2.8)	7 (1.8)	4 (0.8)	0 (0.0)
Grade 6-9	181 (15.7)	49 (12.3)	27 (5.0)	10 (4.5)
Grade 10-12	860 (74.5)	333 (83.5)	454 (84.1)	188 (84.3)
Univ/college/grad	81 (7.0)	10 (2.5)	55 (10.2)	25 (11.2)
Employment				
No, unemployed	1000 (86.7)	352 (88.2)	463 (85.7)	196 (87.9)
Yes, part-time	78 (6.8)	25 (6.3)	31 (5.7)	11 (4.9)
Yes, full-time	76 (6.6)	22 (5.5)	46 (8.5)	16 (7.2)
Whom you live with				
Alone	45 (3.9)	12 (3.0)	25 (4.6)	8 (3.6)
Spouse	52 (4.5)	15 (3.8)	15 (2.8)	8 (3.6)
Family/relatives	859 (74.4)	315 (78.9)	375 (69.4)	160 (71.8)
Roommate/friend	103 (8.9)	34 (8.5)	28 (5.2)	12 (5.4)
Partner	95 (8.2)	23 (5.8)	97 (18.0)	35 (15.7)
Married				
No	1056 (91.5)	369 (92.5)	507 (93.9)	211 (94.6)
Yes	98 (8.5)	30 (7.5)	33 (6.1)	12 (5.4)

	Bloemfontein		Rustenburg	
	Cross-sectional N = 1154	Prospective N = 399	Cross-sectional N = 540	Prospective N = 223
Contraceptive use				
None	540 (46.9)	182 (45.6)	157 (29.1)	58 (26.0)
Oral	55 (4.8)	20 (5.0)	24 (4.4)	19 (8.5)
Injectable	384 (33.3)	138 (34.6)	132 (24.4)	65 (29.2)
IUD	2 (0.2)	0 (0.0)	1 (0.2)	1 (0.5)
Condoms	148 (12.9)	52 (13.0)	201 (37.2)	80 (35.9)
Other	23 (2.0)	7 (1.8)	1 (0.2)	0 (0.0)
Missing	2 (0.2)	0 (0.0)	24 (4.4)	0 (0.0)
STI signs/symptoms				
Vaginal discharge	155 (13.4)	52 (13.0)	154 (28.5)	59 (26.5)
Painful urination	103 (8.9)	31 (7.8)	153 (28.3)	57 (25.6)
Lower abd. pain	115 (10.0)	39 (9.8)	202 (37.4)	82 (36.8)
Vaginal itching	170 (14.7)	60 (15.0)	155 (28.7)	63 (28.5)
Dyspareunia	104 (9.0)	35 (8.8)	121 (22.4)	50 (22.4)
Vaginal sore	27 (2.3)	12 (3.0)	142 (26.3)	66 (29.6)
No. sex partners in last month				
0	53 (4.6)	18 (4.5)	85 (15.7)	40 (17.9)
1	362 (31.4)	120 (30.1)	400 (74.1)	155 (69.5)
2+	739 (64.0)	261 (65.4)	55 (10.2)	28 (12.6)
Vaginal sex w/o condom past week w primary partner	638 (55.3)	202 (50.6)	190 (35.2)	78 (35.0)
Vaginal sex w/o condom past week w other partner	150 (13.0)	50 (12.5)	8 (1.5)	3 (1.3)

The baseline features of women who entered the prospective studies were broadly similar to those of the source screening populations at both sites (Table 1), and there was little indication of selection for a particular subgroup into the prospective cohort.

HIV prevalence

In Bloemfontein cross-sectional screening, the HIV prevalence was 21.2% (95% confidence interval [CI] 18.9-23.6) overall: 13.4% in those 18-24 years; 38.1% in those 25-29 years; and

37.1% in women aged 30-35 years (Table 2). In Rustenburg, the overall HIV prevalence at screening was 23.5% (95% CI 19.9-27.1) among women, ranging from 18.7% in those age 18-24 to 31.8% in those age 25-29 to 37.7% in those age 30-35 (Table 2).

Table 2. HIV Prevalence among Women in Cross-sectional Screening by Age Group and Research Site

	Bloemfontein	Rustenburg
Age 18-24		
Eligible Population[1], N	776	369
HIV positive, N	104	69
Prevalence (95% CI[2])	13.4% (11.0-15.8)	18.7% (14.7-22.7)
Age 25-29		
Eligible Population[1], N	226	110
HIV positive, N	86	35
Prevalence (95% CI[2])	38.1% (31.7-44.4)	31.8% (23.1-40.5)
Age 30+		
Eligible Population[1], N	143	61
HIV positive, N	53	23
Prevalence (95% CI[2])	37.1% (29.1-45.0)	37.7% (25.5-49.9)
TOTAL		
Eligible Population[1], N	1145	540
HIV positive, N	243	127
Prevalence (95% CI[2])	21.2% (18.9- 23.6)	23.5% (19.9- 27.1)

[1] Eligible and had HIV test result
[2] Approximate confidence interval

Factors associated with prevalent HIV

Three baseline factors were significantly associated with prevalent HIV infection at both study sites (Table 3): age 30 or older compared with 18-24; less versus more educational attainment; and living with a child versus not living with child. Self-reported signs or symptoms of STI were associated with HIV in Bloemfontein only, and non-use of contraception versus use of condoms

was associated with HIV in Rustenburg only. Sexual behavior factors were not significantly associated with prevalent HIV infection.

Table 3. Multivariable Logistic Regression Analysis of Factors Associated with Prevalent HIV among Women Screened in Bloemfontein and Rustenburg, South Africa

	Bloemfontein			Rustenburg		
Factor	**Odds Ratio**	**95% CI**		**Odds Ratio**	**95% CI**	
Age 18-24 vs 30+	**0.389**	**0.243**	**0.623**	**0.485**	**0.284**	**0.826**
Age 24-29 vs 30+	1.222	0.766	1.950	0.844	0.502	1.419
Education: Less than High school	**2.240**	**1.552**	**3.233**	**1.979**	**1.099**	**3.563**
Employed: yes	0.913	0.587	1.419	0.790	0.509	1.227
Living with child	**1.569**	**1.074**	**2.290**	**2.078**	**1.398**	**3.088**
Married	0.900	0.541	1.498	0.664	0.304	1.450
STI sign/symptom	**2.081**	**1.472**	**2.940**	1.071	0.729	1.573
Contraception: no method vs condom / other	1.395	0.874	2.229	**2.056**	**1.319**	**3.203**

	Bloemfontein			Rustenburg		
Factor	**Odds Ratio**	**95% CI**		**Odds Ratio**	**95% CI**	
Contraception: effective vs condom / other	0.777	0.474	1.273	1.321	0.827	2.111
More than 1 partner	1.250	0.594	2.632	1.425	0.863	2.354
1 or more new partner	1.168	0.853	1.598	1.136	0.724	1.783
More than 1 different partner	0.809	0.564	1.161	0.836	0.504	1.387
1 or more sex without condom with primary partner last month	1.279	0.917	1.786	1.165	0.776	1.748
1 or more sex without condom with any other partner last month	1.067	0.622	1.831	1.224	0.444	3.379
Anal sex with primary partner	0.828	0.407	1.686	0.974	0.318	2.984
Anal sex with any other partner	0.660	0.183	2.383	1.333	0.153	11.577

Significant factors are in **bold-face.**

HIV incidence

Nine seroconversions were observed during the prospective study in the Bloemfontein cohort, an overall incidence of 5.5 per 100 PY (95% CI 2.5-10.4; Table 4). The incidence was 5.7 per

100 PY (95% CI 2.3-11.7) in women 18-24 years, 4.2 per 100 PY (95% CI 0.1-23.5) among women 25-29 years, and 5.8 per 100 PY (95% CI 0.2-32.2) in the sparse 30-35 years age stratum.

Two seroconversions were detected in the Rustenburg cohort for an overall incidence of 3.0 per 100 PY (95% CI 0.4-10.8; Table 4). Both seroconversions were in the 18-24 year age group, and the incidence in that younger age range was 4.0 per 100 PY (95% CI 0.5-14.5).

Table 4. HIV Incidence among Women in the Prospective Study at the Bloemfontein and Rustenburg Sites

	Bloemfontein	Rustenburg
N with HIV test result during follow-up	391	194
Confirmed HIV seroconversions	9	2
Person-years of follow-up	164.4	67.1
Incidence rate[1] (95% CI[2])	5.5 (2.5-10.4)	3.0 (0.4-10.8)

[1]Per 100 person-years
[2]Exact confidence interval

Ten of the eleven incident HIV infections at the two sites clearly occurred after enrollment in the prospective phase. One seroconversion in Bloemfontein was more problematic; rapid tests were negative at cross-sectional testing and no virus was detected in that stored specimen by PCR, but Western blot results were positive for the stored baseline specimen and were persistently blot-positive until seroconversion at the three-month visit. After excluding that Bloemfontein participant, the re-calculated Bloemfontein incidence rate was 4.9 per 100 PY (95% CI 2.1-9.6).

Factors associated with incident HIV

With nine new infections in the Bloemfontein cohort, we performed univariate proportional hazards regression with the same factors included in the analysis of risk factors for prevalent infection (above). No factor was significantly associated with incident infection. The strongest

association with incident HIV was found for one or more unprotected coital acts with a non-primary partner in the last month (hazard ratio = 6.7; p=0.07).

Pregnancy

The prevalence of pregnancy at the time of cross-sectional screening was 6.5% (95% CI 5.0-7.9) in Bloemfontein and 8.6% (95% CI 6.2-10.9) in Rustenburg. (Further analysis of the Rustenburg pregnancy results will appear elsewhere.) In Bloemfontein, the pregnancy prevalence in screened women ranged from 11.0% (95% CI 8.5-14.0) in those not using a method to 3.5% (95% CI 1.3-7.5) in condom users and 2.1% (95% CI 0.9-3.9) in users of effective methods (hormonal and IUDs). The Bloemfontein pregnancy incidence was 16.4 per 100 PY (95% CI 10.8-23.9), and ranged from 27.6 per 100 PY (95% CI 17.1-42.2) in women not using a method at baseline to 12.4 per 100 (95% CI 2.6-36.4) in condom users and 4.7 per 100 (95% CI 1.0-13.7) in users of effective methods (hormonal or IUD).

DISCUSSION

HIV prevalence in Bloemfontein's Free State Province has been close to 30% in antenatal surveillance for several years. Prevalence was lower in this study, at 21.2%, yet we detected a high incidence rate of 5.5 per 100 PY in the overall cohort. We observed similar HIV incidence across the age spectrum in the Bloemfontein cohort, contrary to national estimates (8). The HIV prevalence among Rustenburg women in the cross-sectional screening was 23.5%, again lower than recent antenatal surveillance data from the area, which could be explained by the young average age of the cohort screened. The prospective incidence rate in the Rustenburg cohort was 3.0 per 100 PY, and higher among younger women (4.0 per 100 PY in 18-24 year olds).

National household survey data in South Africa show that HIV prevalence has stabilized, and suggest that the incidence estimated from nationally representative surveys has declined in younger age groups (8). But the overall estimated incidence remained 1.3 per 100 uninfected persons of reproductive age from 2005-2008 (8). Others have described a pattern of stable prevalence in South African antenatal surveillance and population-based surveys coupled with substantial incidence rates in defined cohorts (9). In the relatively few studies that have directly measured HIV incidence, it has been distressingly high in both urban and rural sites, despite multiple follow-up contacts, ongoing risk-reduction counseling, and condom promotion and provision. Between 2001 and 2004, a representative cohort in one part of rural Limpopo Province had an HIV incidence of 4.9 per 100 person-years among women, despite self-

reported increases in condom use during that period (10). In the HPTN 055 cohort study, the two South African sites, both located in Kwa-Zulu Natal Province, had the highest HIV incidence rates in 2003-04 (Durban 5.3 per 100 PY; Hlabisa 6.2 per 100 PY) (11). In 2004-07, the HIV incidence rate was 6.4 per 100 PY and 6.5 per 100 PY in an urban and a rural cohort in KwaZulu-Natal, respectively (9). Three recent randomized trials of ineffective prevention products have also reported substantial HIV incidence at their South African sites (12-14). Finally, a cohort study conducted 2007-09 at two sites in Northwest Province and Western Cape Province for capacity-building purposes reported 12-month incidence rates of 6.0 per 100 PY and 4.5/100 PY, similar to the rates reported here (15).

Older participants had a higher risk of prevalent HIV infection in our study than younger, reflecting their longer potential for exposure to HIV. Lesser education was also associated with baseline HIV infection, as has been found in other South African studies (10, 16). Other factors were not consistently associated with baseline infection across the sites, which may stem from unmeasured sexual network factors, and misclassification error resulting from self-report. In fact, the striking differences in the self-reported risk behaviors between these two cohorts suggest that some self-reporting bias may have occurred. Bloemfontein participants reported high rates of multiple partnering (about two-thirds of the women), compared with national data on South African women aged 15-49 (3.7% in 2008) and data on men and women in the Free State in the same 2008 survey (14.6% in reference 17). In contrast, reports of multiple partnering at the Rustenburg site were close to national averages (12-14%) and similar to a prior representative household survey in Rustenburg (18).

One weakness of our analysis is that, partly due to the relatively short follow-up period, there were too few seroconversion events to do multivariable proportional hazards regression, and our univariate regression results for incident HIV infections were necessarily inconclusive. A limitation of the analysis of risk factors for prevalent HIV infections is that the behaviors reported at screening may not have reflected behaviors present at the time of infection. Lack of STI diagnostic testing precluded evaluating associations with other infections.

Perhaps the greatest study weakness relates to the validity of self-reported sexual behaviors, both for purposes of determining eligibility as well as for assessing risk during follow-up. The inaccuracies in self-reported sexual behavior during research interviews have been amply documented and are not unique to these studies. Although we attempted to keep the eligibility

factors secret from participants and outreach staff, we do not know if volunteers (both eligible and ineligible) guessed those factors and shared them with other women in the community. There may have been an awareness among women at the Bloemfontein site in particular to over-report risky behaviors to gain entry to the study: the eligibility rate was very high there, and risk behaviors far exceeded national estimates. Alternatively, recruiters at the Bloemfontein, but not the Rustenburg, site may have simply tapped into a sub-group of women with particularly risky behavior. During baseline and follow-up interviews with participants, however, we did not observe strong associations between those same behavioral factors and HIV infection.

Our study cohorts cannot be considered representative of young women in the two cities. Our behavioral eligibility criteria selected for a group of women with higher than average risk behaviors. The Rustenburg site offered the opportunity to demonstrate the impact of these selection factors: a representative household survey had been done there in 2008, one year before our study commenced. Our study cohort was younger, better educated, less likely to be employed, and less likely to be married or live with a steady partner than all Rustenburg community residents. Most critically, only 3.4% of women in the household survey reported multiple sexual partners in the past three months, compared with over 10% of women screened in our study who reported multiple partners in the past one month. So we make no claims for representativeness: our project deliberately set out to identify and follow a cohort with higher than average risk behaviors, in order to demonstrate the suitability of the sites for future prevention research.

Countervailing study strengths include the longitudinal design with high retention rates. Classification of the HIV outcome was highly accurate, using multiple monthly rapid tests confirmed by later PCR testing. Testing of stored baseline specimens allowed us to pinpoint seroconversion and rule out acute pre-study infections. Finally, direct prospective measurement of HIV infection circumvented the lingering issues with the accuracy of cross-sectional incidence estimation.

The high HIV prevalences and incidence rates in these cohorts in two smaller South African cities should spur vigorous HIV prevention efforts. The need for HIV care and treatment in these areas is clear, and while care is available, Free State Province experienced stock-outs of HIV antiretroviral drugs during the study (19). Our results also highlight the success of the collaborative capacity-building SIDI projects: these sites are highly suitable and ready for HIV

prevention research and programming. Both sites added staff and were provided broad research training, and these new skills were immediately applied in a rigorous clinical research study. Dealing with local ethics committees and community stakeholders, recruiting and enrolling potentially higher-risk women, administering informed consent, bringing participants back for regular study visits, giving ongoing risk-reduction and HIV counseling, and making referrals for continued health care, all within the context of Good Clinical Practice, mimicked future prevention trials. At the conclusion of its study, JOSHA in Bloemfontein was selected to implement an oral HIV prophylaxis trial. The Aurum Institute in Rustenburg is currently conducting a trial of a vaginal microbicide, along with other behavioral HIV studies. The national research capacity has been boosted and diffused to new sites outside the larger cities, which can only be a boon for future HIV and other clinical research in South Africa.

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PJF, MHL, JL, CC, PLC and CS contributed to the design of the study and its essential documents. MHL, JL, and CC led data collection. PJF, MHL, JL, PLC, CS and SF contributed to the analysis and interpretation of the data. PJF, MHL, JL, CC, and SF drafted the article. All authors approved the final version of the manuscript. The authors declare no competing interests.

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4-6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5, 8
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-7
Bias	9	Describe any efforts to address potential sources of bias	5, 10
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7-8
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7-8
		(*b*) Describe any methods used to examine subgroups and interactions	NA
		(*c*) Explain how missing data were addressed	NA
		(*d*) If applicable, explain how loss to follow-up was addressed	NA
		(*e*) Describe any sensitivity analyses	NA

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	8
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8-10
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) Summarise follow-up time (eg, average and total amount)	8
Outcome data	15*	Report numbers of outcome events or summary measures over time	11, 14
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	12-13
		(*b*) Report category boundaries when continuous variables were categorized	12
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11, 14
Discussion			
Key results	18	Summarise key results with reference to study objectives	15-16
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	16-17
Generalisability	21	Discuss the generalisability (external validity) of the study results	15-16
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	18

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.