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Seroepidemiology of *Trichomonas vaginalis* in rural women in Zimbabwe and patterns of association with HIV infection

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SUMMARY

Serological assays using dried blood spots from 5221 women in rural areas of eastern Zimbabwe were used to assess the epidemiology of *Trichomonas vaginalis* infection, and its association with HIV. Antibodies to *T. vaginalis* and to HIV were detected by enzyme immunoassays. Behavioural and demographic data were collected by confidential questionnaires. In total, 516 (9·9%) women were seropositive for *T. vaginalis* and seroprevalence increased with age among younger women. Divorced, widowed and single women were more likely to be seropositive. After controlling for age, seropositivity was significantly associated with being sexually active, having multiple sex partners, having a partner who had multiple sex partners, and having a new sex partner in the past year. Seropositivity was associated with a recent history of genital discharge. Overall, 208 (40·3%) *T. vaginalis*-positive samples were also positive for HIV, compared with 1106 (23·5%) *T. vaginalis*-negative samples (age and sex adjusted OR 2·11, 95% CI 1·74–2·55, $P<0·001$). There was increased risk for being HIV-positive amongst *T. vaginalis*-seropositive women regardless of residence, employment or education. In a logistic regression controlling for common risk factors, the association remained significant. *T. vaginalis*-seropositive young women with a history of genital discharge were much more likely to be HIV-positive than women who were *T. vaginalis*-seronegative and had no history of discharge (OR 6·08, 95% CI 2·95–12·53). Although a causal relationship cannot be assumed, detection and treatment of trichomoniasis may be important in strategies to reduce HIV transmission through sexually transmitted infection control.

INTRODUCTION

Trichomonas vaginalis infection is among the most frequently occurring sexually transmitted infections (STIs), and is associated with vaginitis, urethritis and cervicitis and with adverse outcomes in pregnancy [1]. In Zimbabwe, the prevalence of trichomoniasis may be as high as 50% in women attending genitourinary clinics [2], while asymptomatic carriage of *T. vaginalis* has been shown in approximately 10% women attending family-planning clinics [3]. Normal methods of detection – microscopy and/or culture of vaginal or

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urethral swabs – are not appropriate for community investigations. Techniques such as polymerase chain reaction assays have been used with self-collected specimens [4], but these are expensive and are not widely available in developing country settings. Information on the epidemiology of trichomoniasis infection in the general population is, therefore, limited. In a rural area of Uganda, a population-based study in which women used self-administered vaginal swabs, found a prevalence of infection of 47% [5].

Epidemiological and biological studies have demonstrated a significant role for STIs as co-factors facilitating HIV transmission. The success of a community-based STI detection and treatment programme in reducing HIV transmission lends support to this hypothesis [6]. Inflammation of the urogenital tract both increases viral shedding and increases susceptibility to HIV infection [7] and untreated STIs have, therefore, been suggested as contributing significantly to the high prevalence of HIV in sub-Saharan Africa [8]. Chronic asymptomatic infections that go undetected and untreated may also be important factors in HIV transmission, and infection with *T. vaginalis* may be important in this regard [9]. Studies in Malawi have shown that urethritis in men is associated with increased levels of HIV in semen, and treating urethritis reduces these levels [10]. Elsewhere in Africa a programme of community mass treatment for common STIs, including trichomoniasis, showed no major impact on HIV seroincidence [11]. There are, however, a number of explanations for this, including the lack of treatment for Herpes simplex infection, and the continued prevalence of trichomoniasis and symptoms of urethritis, despite the intervention.

In most cases, an STI is detected by microscopy and culture, methods that are applicable only to current, even if asymptomatic, infection. Detection of HIV infection, however, is usually carried out using serology and antibodies to HIV may only be found weeks or months after the event leading to infection occurred. As a consequence, attempts to identify an epidemiological association between current infection events, such as symptomatic STI episodes, and past infection events, such as HIV, may be difficult to establish. Serological assays, however, are indicative of the entire past history of exposure to specific antigens, and so may be more useful in detecting these associations.

Recently we evaluated an enzyme immunoassay (EIA) that detects exposure to trichomonal infection, and found it had a sensitivity $>90\%$ in men and women at high and low risk of having had a *T. vaginalis* infection [12]. Here, we present data from a subsequent evaluation of this EIA in detecting anti-trichomonal antibody in dried blood spot (DBS) specimens collected in community studies. We describe the seroepidemiology of *T. vaginalis* infection in women and the association between seropositivity for *T. vaginalis* and HIV in rural communities in eastern Zimbabwe.

MATERIALS AND METHODS

The samples for the current study were collected in the course of broader programmes of research on the epidemiology, control and impact of HIV in men and women in the Mutasa, Makoni and Nyanga districts of Manicaland, Zimbabwe's eastern province. The study design and finger-prick DBS sample collection procedures were approved by both the Institutional Review Board of the Biomedical Research & Training Institute, and by the Medical Research Council of Zimbabwe and are reported elsewhere [13]. All research participants gave informed written consent to serological screening for STI and HIV. Samples were labelled with reference numbers and tests were conducted anonymously. Parallel services for STI treatment and voluntary counselling and testing for HIV infection were made available. Sociodemographic and epidemiological data were collected by standard questionnaire in a confidential setting. Data on sexual behaviour were collected using an informal, confidential, voting interview procedure [14].

Specimen collection and processing procedures

In addition to DBS, whole blood specimens were collected from a subsample of 102 men and women participating in the main study. These paired samples were used to evaluate DBS samples for serological testing for HIV and *T. vaginalis*. Serum was separated from the whole blood samples after centrifugation and was stored at $-20\,^{\circ}C$ in 250-μl aliquots. The blood spots were collected on Whatman No. 1 filter papers. The filter papers were air dried and stored at room temperature in individual paper packets. In the laboratory, a 10-mm diameter circle was cut from each blood spot and placed into a tube with 400 μl PBS. After incubation overnight at $4\,^{\circ}C$, the eluate was removed and used for serological tests. The DBS eluates were regarded as a 1:20 dilution of

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serum [15] for the purposes of subsequent serological testing. Sera were tested immediately after thawing and DBS eluates were used within 24 h of preparation.

In the main study, DBS samples only were obtained from 4166 men (aged 17–49 years) and 5299 women (aged 15–49 years). These donors were residents of small towns, roadside trading centres, large-scale commercial farming estates (mainly tea, coffee and forestry plantations), and ('communal') subsistence farming areas. Individuals who wished to receive HIV test results provided a separate whole blood sample that was tested independently of the DBS eluate. This separate examination was also used for purposes of quality control.

Laboratory investigations

For detection of anti-trichomonal antibodies, we followed the EIA protocol described previously using an antigen derived from a *Mycoplasma*-free isolate of *T. vaginalis* [12]. Approximately 30 000–50 000 cells were inoculated into the wells of microtitre plates, which were then air dried and fixed with methanol. The test has been shown to have high sensitivity (95%) and specificity (85%) in women with a current *T. vaginalis* infection, and also to detect probable past exposure to trichomonal antigens. Specimens that gave borderline results (OD = 0·4–0·5) were re-tested and, if still borderline, were regarded as indeterminate for the purpose of this analysis and excluded.

Antibodies to HIV-1 and HIV-2 were detected in both serum and DBS eluates using an EIA (ICL HIV-1/2 dipstick, Bangkok, Thailand) that has been evaluated under local conditions [16]. Sera that gave borderline results were subjected to a second assay for confirmation (Abbott 3rd Generation HIV-1/2 EIA, USA or Genelavia MIXT HIV-1/2, Sanofi Diagnostics, Pasteur SA, France).

Data analysis

Sociodemographic, epidemiological and sexual behaviour data collected in the population survey together with results from the serological tests were entered and validated using SPSS-PC (SPSS Inc., Chicago, IL, USA). Statistical analyses, including estimation of adjusted odds ratios for HIV infection given positive serological test results for exposure to *T. vaginalis* infection using logistic regression, were conducted in STATA version 5 (StataCorp, TX, USA).

RESULTS

Evaluation of EIAs for *T. vaginalis* and HIV infections when applied using DBS

Concordant *T. vaginalis* EIA test results were obtained in 100/102 (98%) of the paired serum and DBS samples. The test gave 14 (13·7%) positive results in the serum samples, 12 (85·7%) of which were also positive when the DBS eluates were tested. In both discrepant cases, the serum OD value was 0·5, the lower limit of positivity in our laboratory. Twenty-nine (28·4%) serum specimens were positive for HIV antibodies using the ICL dipstick EIA. In 28 (96·6%) of these cases, the same assay also gave positive results with the DBS samples. The Abbott EIA gave positive results with 31 sera (30·3%), and 29 (93·5%) of the matched DBS samples also gave positive HIV test results. In one case, the serum was repeatedly positive but the DBS eluate was negative by both ICL dipstick and Abbott EIA. This serum was also positive by Genelavia and the DBS eluate result was regarded as a false-negative. In each case where the serum was negative, the DBS eluate was also negative.

Population seroepidemiology of *T. vaginalis* antibodies

The prevalence of *T. vaginalis* and HIV in the community was determined from DBS eluates only. The *T. vaginalis* EIA gave indeterminate results in 16 (0·4%) men and 78 (1·5%) women, and these were excluded from further analysis. Within the remainder, the prevalence of antibodies to *T. vaginalis* was 1·1% (47/4150) in men and 9·9% (516/5221) in women and antibodies to HIV was 19·2% (797/4150) in men and 25·2% (1316/5221) in women. Because of the very low number of *T. vaginalis*-seropositive men, they were excluded from subsequent analysis.

Infection with *T. vaginalis* occurred early in women with 10% of female respondents being seropositive before the age of 20 years (Fig.). Seroprevalence in women peaked in the late twenties but remained high throughout the reproductive age-range.

The highest seroprevalence was found amongst residents of roadside trading centres – with lower prevalence levels being found in commercial farming estates, small towns and subsistence farming areas (Table 1). Seroprevalence was higher in respondents with no formal education and in subsistence farmers, and was also low in students. However, these patterns probably reflect age differences in socioeconomic status and the marked increases in education levels

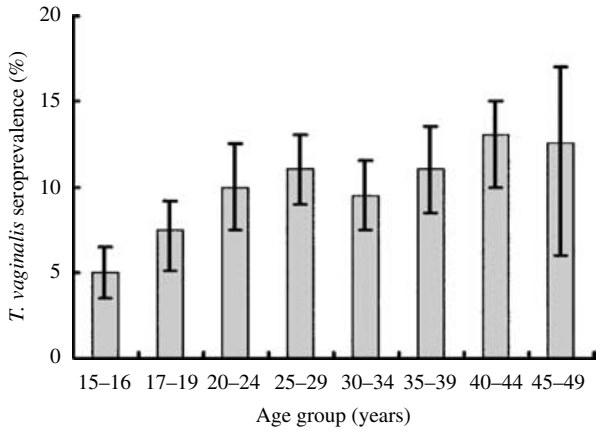

Fig. *T. vaginalis* seroprevalence with 95% confidence intervals for women aged 15–49 years.

that have taken place in Zimbabwe since independence in 1980.

Women who were currently divorced, widowed or sexually active but not yet married were more likely to be seropositive. A number of women who reported having not yet started sex were found to be seropositive for *T. vaginalis*. This may indicate inaccuracy in responses to questions concerning sexual behaviour, especially as the HIV seroprevalence in this group was also implausibly high. Qualitative studies in the project areas and evaluations of the sexual behaviour data using other biomarkers have shown that misreporting of onset of sexual activity can occur [17].

After controlling for age, a positive *T. vaginalis* serological test was associated with onset of sexual activity, with having multiple lifetime sexual partners, with having had a new sexual partner in the preceding year, and with having a regular partner who has multiple sexual partners (Table 1). The chances of having a positive *T. vaginalis* test result rose with increasing numbers of lifetime sexual partners (age-adjusted OR 1·02, 95% CI 1·00–1·04, $P=0·088$).

There was evidence for an association between a history of genital discharge and *T. vaginalis* infection, but the association was only apparent in those women reporting a discharge within the last year rather than longer ago. There were 316/3478 (9·1%) women with no history of a discharge who were seropositive, compared to 132/1115 (11·8%) women reporting a discharge in the previous year (OR 1·34, 95% CI 1·08–1·68, $P=0·007$) and 11/180 (6·1%) women reporting discharge more than 12 months previously (OR 0·65, 95% CI 0·35–1·25, $P=0·173$). For this analysis, women reporting lower abdominal pain in the past year, but who did not report a history of genital discharge were included in the reference category. If these are excluded completely, the effects noted here strengthen further: the age-adjusted odds ratios for a positive *T. vaginalis* EIA result in women with a recent history of discharge increase to 1·36 (95% CI 1·09–1·70, $P=0·006$).

Table 1. T. vaginalis (*TV*) *seroprevalence by sociodemographic status and sexual behaviour*

Characteristic	TV %	*n*	OR	*P*
Location				
Subsistence farm	7·1	1996	1	
Small town	8·0	777	1·13	0·433
Commercial farm	9·3	1397	1·33	0·021
Trading centre	17·3	1051	2·73	<0·001
Education				
None	16·8	208	1	
Primary	11·1	2239	0·62	0·015
Secondary or higher	8·4	2773	0·45	<0·001
Employment				
Subsistence farming	13·5	341	1·45	0·041
Married – cohabiting	9·7	1433	1	
Married – living apart	8·5	1494	0·94	0·616
Divorced or widowed	14·7	1009	1·60	<0·001
Beer-hall attendance last month				
Self – yes	11·9	202	1·25	0·312
Self – no	9·7	4991	1	
Regular partner – yes	9·8	1845	1·10	0·447
Regular partner – no	9·3	992	1	
Lifetime sexual partners				
0	4·8	942	0·56	<0·001
1	9·3	2677	1	
2	13·7	795	1·54	<0·001
⩾3	15·1	720	1·62	<0·001
New partner in last year				
No (but sexually active)	10·0	2729	1	
Yes	13·9	771	1·54	<0·001
Partners in the last month				
0 (but previously active)	13·3	1551	1	
1	9·9	2579	0·72	0·001
⩾2	8·8	80	0·63	0·251
Regular partner has non-marital partner in the last year?				
0	8·6	973	1	
1	10·8	481	1·27	0·197
⩾2	12·6	286	1·44	0·217
Time since last had sex				
>1 year (but started)	13·6	722	1	
1–12 months	12·8	963	0·95	0·716
<1 month	9·6	2592	0·69	0·003
Last partner was				
Regular	9·6	2335	1	
Casual	12·8	188	1·42	0·133

OR, Odds ratio for *T. vaginalis* seropositivity in relation to reference category in each case.

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Table 2. *Prevalence of HIV infection in women by* T. vaginalis (*TV*) *serology*

	TV positive		TV negative				
Sociodemographic group	HIV % positive	*n*	HIV % positive	*n*	OR	(95% CI)	*P*
Residence							
All sites	40·3	516	23·5	4705	2·19	(1·82–2·65)	<0·001
Small town	64·5	62	41·7	715	2·54	(1·48–4·37)	0·001
Commercial farm	46·2	130	22·8	1267	2·90	(2·01–4·19)	<0·001
Trading centre	31·3	182	20·4	869	1·78	(1·25–2·54)	0·001
Subsistence farm	35·9	142	18·5	1854	2·47	(1·72–3·55)	<0·001
Age group (years)							
15–19	31·3	80	5·3	1155	8·15	(4·76–13·97)	<0·001
20–24	45·5	101	24·1	905	2·64	(1·73–4·01)	<0·001
25–29	57·9	95	37·4	738	2·30	(1·49–3·55)	<0·001
30–34	37·3	59	39·5	582	0·91	(0·52–1·58)	0·738
35–39	42·7	75	30·7	592	1·68	(1·03–2·74)	0·039
40–44	26·7	86	18·7	589	1·59	(0·94–2·68)	0·081
45–49	25·0	20	20·8	144	1·27	(0·43–3·76)	0·671
Marital status							
Single – not started sex	15·6	45	1·7	897	10·83	(4·17–28·12)	<0·001
Single – started sex	54·3	46	22·5	293	4·09	(2·16–7·78)	<0·001
Married – live together	30·9	139	24·6	1293	1·37	(0·94–2·01)	0·102
Seasonally separated	30·7	137	20·8	1357	1·69	(1·15–2·48)	0·008
Permanently separated	64·9	94	45·7	516	2·19	(1·86–3·26)	0·001
Widowed	55·6	54	55·2	344	1·01	(0·57–1·80)	0·979
Education level							
None	37·1	35	29·5	173	1·41	(0·66–2·02)	0·372
Primary	42·2	249	26·9	1990	1·98	(1·51–2·59)	0·001
Secondary or higher	38·8	232	20·5	2540	2·46	(1·86–3·26)	<0·001
Employment status							
Subsistence farmer	44·6	193	26·2	1468	2·27	(1·67–3·08)	<0·001
Formal sector	55·4	65	28·4	656	3·13	(1·87–5·25)	<0·001
Unemployed	35·8	229	25·2	2107	1·66	(1·24–2·21)	0·001
Student	11·5	26	1·1	466	12·03	(2·71–53·43)	<0·001

OR, Odds ratio for HIV infection.

Association between *T. vaginalis* and HIV infections

There was a strong overall association between seropositivity for *T. vaginalis* and HIV, with 208/516 (40·3%) *T. vaginalis*-positive samples being HIV-positive, compared to 1106/5221 (23·5%) of the *T. vaginalis*-negative samples (age adjusted OR 2·11, 95% CI 1·74–2·55, $P<0·001$).

Increased risk for being HIV-positive in women with positive serology for *T. vaginalis* was found regardless of residence, employment or educational level (Table 2). The association was strongest at young age, almost certainly reflecting selection for early sexual activity, and relatively weak (but still present) at age $\geqslant$30 years. The latter probably contributes towards the absence of an association amongst widows and women with no formal school education, as most of these would be older women.

The results of logistic regression analyses of the association between positive serologies for *T. vaginalis* and HIV infections after controlling for common risk factors is shown in Table 3. The results of a separate analysis for young women (<25 years) are presented as they shed some light on the possible strength of this association at ages where prevalence is likely to reflect incidence most closely for both infections.

These results show that area of residence, age, marital status, employment sector, beer-hall attendance and several specific aspects of sexual behaviour including cumulative number of lifetime sexual partners and regular partner's number of non-marital partners, are all important risk factors for HIV as well

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Table 3. *Odds ratios for HIV infection in women with positive* T. vaginalis (*TV*) *serology in the presence and absence of symptoms, adjusted for sociodemographic and behavioural risk factors*

	All women (15–49 years) ($n=4183$)			Young women only (15–24 years) ($n=1274$)	
Characteristic	OR (95% CI)	*P*		OR (95% CI)	*P*
STD symptoms and TV					
TV positive, genital discharge ever	2·89 (2·00–4·15)	<0·001		6·08 (2·95–12·53)	<0·001
TV positive – no genital discharge	1·77 (1·33–2·35)	<0·001		3·18 (1·92–5·26)	<0·001
TV negative – genital discharge ever	1·51 (1·28–1·78)	<0·001		1·52 (1·09–2·10)	0·013
TV negative, no genital discharge	1			1	
Location					
Small town	2·73 (2·19–3·40)	<0·001		2·37 (1·92–5·26)	<0·001
Commercial farm	1·22 (1·00–1·50)	0·053		1·37 (0·93–2·01)	0·108
Trading centre	0·98 (0·79–1·21)	0·862		0·77 (0·50–1·21)	0·258
Subsistence farm	1			1	
Age group (yr)			Age (yr)		
15–19	1		15	1	
20–24	1·69 (1·23–2·33)	0·001	16	2·89 (0·31–27·14)	0·352
25–29	2·41 (1·74–3·33)	<0·001	17	3·02 (0·36–25·65)	0·311
30–34	2·19 (1·56–3·08)	<0·001	18	2·71 (0·32–23·08)	0·362
35–39	1·56 (1·10–2·21)	0·012	19	4·79 (0·5–38·85)	0·142
40–44	0·74 (0·52–1·07)	0·111	20	0·79 (0·34–22·78)	0·339
45–49	0·67 (0·40–1·12)	0·127	21	6·20 (0·77–50·20)	0·087
			22	8·04 (1·00–64·64)	0·050
			23	5·71 (0·71–45·74)	0·101
			24	9·25 (1·15–74·40)	0·037
Marital status					
Single	1			1	
Married – cohabiting	0·90 (0·64–1·26)	0·534		1·01 (0·62–1·66)	0·953
Married – live apart seasonally	0·93 (0·66–1·31)	0·681		0·76 (0·46–1·27)	0·299
Divorced or separated	1·94 (1·40–2·70)	<0·001		1·34 (0·82–2·21)	0·243
Widowed	5·01 (3·43–7·32)	<0·001		3·29 (1·34–8·12)	0·010
Employment					
Subsistence farmer	1			1	
Formal sector	0·74 (0·59–0·95)	0·016		0·59 (0·36–0·96)	0·034
Unemployed	0·93 (0·79–1·10)	0·395		0·81 (0·59–1·11)	0·194
Student	0·28 (0·08–0·96)	0·043		0·51 (0·14–1·88)	0·312
Beer-hall attendance in last month					
Not visited a beer-hall	1			1	
Visited a beer-hall	1·38 (0·96–1·97)	0·083		1·41 (0·55–3·59)	0·477
Partner not visited a beer-hall	1			1	
Partner visited a beer-hall	1·23 (1·01–1·50)	0·039		1·65 (1·15–2·37)	0·006
Sexual behaviour					
Total sex partners					
1	1			1	
2	1·79 (1·48–2·16)	<0·001		2·08 (1·44–2·99)	<0·001
3	2·54 (1·94–3·32)	<0·001		2·99 (1·81–4·95)	<0·001
⩾4	3·62 (2·79–4·70)	<0·001		3·20 (1·88–5·45)	<0·001
Regular partner has other partners in last year					
0	1			1	
1	1·11 (0·90–1·36)	0·327		0·92 (0·61–1·37)	0·674
2	1·14 (0·82–1·59)	0·441		1·27 (0·69–2·33)	0·447
⩾3	1·40 (1·00–1·96)	0·050		1·21 (0·68–2·15)	0·509

Age at first sex, partners in last month, time since last sex and education all had small, not significant effects in each age range.

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as *T. vaginalis* in this rural population. However, after controlling for these factors, the strong association between positive *T. vaginalis* and HIV serologies remains. Women with positive *T. vaginalis* serology coupled with a self-reported history of genital discharge are almost three times as likely to be HIV-positive than those with negative serology and no history of this symptom. However, even those with positive *T. vaginalis* serology but who do not report a history of genital discharge were found to be at significantly greater risk of having been infected with HIV. Both of these effects were particularly strong in the 15–24 years age group, despite controlling for reported number of lifetime partners, and remained so even when the effect of age at first sex was tested in the regression model.

DISCUSSION

The overall seroprevalence of HIV in this rural cohort was slightly lower than reported in studies of pregnant (33%) [18] or non-pregnant (30–35%) [19] women in Harare. This is consistent with many studies showing small differences in HIV prevalence between urban and rural communities, and such differences have also been noted when comparing small urban sites and rural sites in this study area [12]. Well-recognized risk factors for prevalent HIV include a recent self-reported history of STI and having multiple sex partners. Similar epidemiological patterns were identified using DBS eluates in this rural cohort. This finding and the direct comparison we were able to make between results using serum and DBS eluates on a limited number of participants give us confidence that the results using eluates are a true reflection of HIV serology in this population. Equally, the high level of concordance between the results obtained using DBS eluates and serum samples drawn from the same individuals and the plausibility and external consistency [1, 20] of the epidemiological results encourage us to believe that the *T. vaginalis* results based on DBS eluates are also essentially valid.

Marked differences have been reported in the clinical presentation and natural history of trichomonal infection in men and women [20]. In men, most infections are mild [21] and if untreated the duration of carriage is estimated to be 1–4 months [22]. This may inhibit the production of anti-trichomonal antibody, resulting in the low seroprevalence recorded here and in previous investigations of asymptomatic men [12]. In women, the acute infection is usually symptomatic, but chronic carriage may follow, with an estimated duration of several years, if not for life [22], and most women with a trichomonal infection have antibodies [12]. Both past and current infections are detected by serology, and positive serology may, therefore, relate to both active acute trichomoniasis, to chronic carriage or even to past episodes of trichomoniasis. This may however be an advantage in examinations of epidemiology, or of association with other infections where the time of actual infection is unknown, but where historical data on behaviour is available.

The seroprevalence data presented here are consistent with previous data on the prevalence of active trichomoniasis in asymptomatic women in an urban environment [3], and also are consistent with many aspects of the epidemiology of trichomoniasis. Seroprevalence increased markedly with age in young women, and remained high in older women, a pattern that has previously been described for trichomoniasis using microscopy and culture and has been attributed to the long duration of infection in women (reviewed in ref. [22]). The association between seropositivity and numbers of lifetime sexual partners also is consistent – the presence of an STI with a long duration of infection generally being most strongly correlated with indicators that reflect risk behaviour over similarly long time intervals and vice versa [22].

The strong association between *T. vaginalis* infection and HIV infection seen here in women is not surprising given the long duration of both infections and the common behavioural risk factors for transmission and acquisition. In women, a positive *T. vaginalis* serology may, in fact, be a useful marker for cumulative risk behaviour for (prevalent) HIV infection, particularly at younger ages. Equally, if the EIA used here detects mainly current or recent *T. vaginalis* infection, it is possible that positive serology could be a useful marker for behaviour associated with recent acquisition (incidence) of HIV infection. More data on the kinetics of serological responses to trichomonal antigens would be useful in this context. Whether detecting recent or past infection, *T. vaginalis* serology might be used in the validation of self-reported sexual behaviour data, and indeed, trichomoniasis has been used as an indicator of poor compliance with use of the female condom [23].

The persistence of the association between *T. vaginalis* and HIV infection across different socio-economic and demographic subgroups and the very strong association that remained after controlling for

the common risk factors are suggestive of a causal link. While a biological risk – through promotion of urethral or vaginal discharge containing large numbers of immune cells – may be attributed in the case of symptomatic trichomoniasis, this may not be the case in asymptomatic infection. There is some evidence that even in the absence of acute symptoms there may be chronic inflammation of the urogenital tract [20] and increased potential for the transmission of HIV in men and women with trichomoniasis has been recorded [8, 24]. However, in a cross-sectional analysis such as this, it is not possible without more detailed immunological investigations, to determine whether such a relationship is causal or to assess a direction of causation. Furthermore, despite the precautionary measures that were taken to reduce misreporting, biases in the data on sexual behaviour and/or STI-related symptoms could mean that the full effects of the confounding factors included in the regression models were not captured. Equally there could be other confounding risks, common to both HIV and *T. vaginalis* infection that were not measured in this study.

Intervention studies would be needed to determine whether there is indeed a causal association between *T. vaginalis* infection and HIV. The Rakai study in Uganda [11] failed to show that repeated mass treatments for STI, including the use of metronidazole, impacted on HIV incidence. However, there are number of possible explanations. The occurrence of other STIs, especially herpetic ulcers, that remained untreated, may have masked the impact of the intervention. The continued detection of trichomonal infection (9–11% after intervention), of bacterial vaginosis (46–54% after intervention) and of urethral discharge (6–7% after intervention) suggest that the treatment intervals may have been too long to substantially reduce these conditions, each of which may be a risk factor for HIV infection, within the community.

In a study in Malawi, the inclusion of metronidazole in syndromic management of urethritis in men failed to reduce the prevalence of persistent urethritis, as determined 1 week after treatment, even though the *T. vaginalis* infection was eliminated [25]. Seminal HIV-1 RNA levels were, however, substantially reduced in men treated for trichomoniasis, and 2 weeks after this treatment the levels in the semen of treated men and in controls, without an STI, were comparable [25]. Given that trichomoniasis is probably the most commonly occurring STI in Africa, focusing treatment at this parasite may be a useful strategy in HIV prevention programmes.

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