Infection with *Trichomonas vaginalis* Increases the Risk of HIV-1 Acquisition

R. Scott McClelland,[1,2,3] Laura Sangaré,[2] Wisal M. Hassan,[3] Ludo Lavreys,[2,3] Kishorchandra Mandaliya,[5] James Kiarie,[4] Jeckoniah Ndinya-Achola,[3] Walter Jaoko,[3] and Jared M. Baeten[1]

Departments of [1]Medicine and [2]Epidemiology, University of Washington, Seattle; Departments of [3]Medical Microbiology and [4]Obstetrics and Gynaecology, University of Nairobi, Nairobi, and [5]Coast Provincial General Hospital, Mombasa, Kenya

We conducted a prospective study among women in Mombasa, Kenya, to determine whether *Trichomonas vaginalis* infection was associated with an increased risk of human immunodeficiency virus type 1 (HIV-1) infection. At monthly follow-up visits, laboratory screening for HIV-1 and genital tract infections was conducted. Among 1335 HIV-1–seronegative women monitored for a median of 566 days, there were 806 incident *T. vaginalis* infections (23.6/100 person-years), and 265 women seroconverted to HIV-1 (7.7/100 person-years). Trichomoniasis was associated with a 1.52-fold (95% confidence interval, 1.04–2.24-fold) increased risk of HIV-1 acquisition after adjustment for potential confounding factors. Treatment and prevention of *T. vaginalis* infection could reduce HIV-1 risk in women.

Trichomonas vaginalis infects 173 million people each year worldwide, and 32 million of these infections occur in sub-Saharan Africa [1]. Although it is well accepted that sexually transmitted infections (STIs) in general increase susceptibility to HIV-1 [2], data exploring the association between *T. vaginalis* infection and HIV-1 acquisition have been inconclusive. Among the 7 prospective studies that have evaluated vaginal

trichomoniasis as a risk factor for HIV-1, all found elevated point estimates [2]. However, only 1 study demonstrated a statistically significant increased risk (relative risk, 1.7 [95% confidence interval {CI}, 1.1–2.8]) [3]. The lack of statistical significance in many of the other studies may have been related to sample size constraints. We used data from an 11-year prospective study of female sex workers (FSWs) in Mombasa, Kenya, to test the hypothesis that infection with *T. vaginalis* increases the risk of HIV-1 acquisition in women.

Participants and methods. From February 1993 through March 2004, HIV-1–seronegative FSWs attending a municipal clinic in Mombasa were invited to participate in an open cohort study of risk factors for HIV-1 acquisition. Detailed procedures have been described elsewhere [4]. At enrollment and monthly follow-up visits, a standardized interview was conducted to ascertain medical, gynecological, and sexual history. A physical examination was performed, and specimens were collected for laboratory diagnosis of HIV-1 and STIs.

Women with signs or symptoms of a genital tract infection at an examination visit were given syndromic treatment in accordance with Kenya Ministry of Health guidelines. Syndromic management for vaginal discharge and for suspected pelvic inflammatory disease included oral metronidazole, which is effective as treatment for *T. vaginalis* infection. All women were asked to return for their laboratory results after 1 week. Additional treatment was provided at the results visit if the laboratory findings identified infections, including with *T. vaginalis,* that were not treated syndromically at the time of the examination. Individual HIV-1 risk-reduction counseling and free condoms were provided at each visit. This study was approved by the institutional review boards of the University of Washington and the University of Nairobi.

Screening for HIV-1 was performed using an ELISA (Detect-HIV; Biochem Immunosystems). Positive results were confirmed using a second ELISA (Recombigen; Cambridge Biotech) [5]. Light microscopy of a vaginal wet preparation at ×40 magnification was used to diagnose vaginal trichomoniasis and candidiasis on the basis of identifying motile trichomonads and yeast forms, respectively. Bacterial vaginosis (BV) was diagnosed using microscopic analysis of a vaginal Gram stain [6]. *Neisseria gonorrhoeae* cultures of cervical secretions were performed on modified Thayer-Martin medium. Cervicitis was defined as the presence of an average polymorphonuclear leukocyte count $\geq$30 cells/high-power field of gram-stained cervical secretions.

Analyses were performed using SPSS (version 10; SPSS) and

S-Plus (2000; Mathsoft). All HIV-1–seronegative women who enrolled in the cohort and had at least 1 follow-up visit were included. Women were censored at HIV-1 seroconversion or at their last follow-up visit.

The association between *T. vaginalis* infection and HIV-1 acquisition was evaluated using univariate and multivariate Cox proportional hazards analysis. *T. vaginalis* infection was analyzed as a time-dependent variable. As we have done previously [4], we assumed that the effect of *T. vaginalis* infection on HIV-1 susceptibility would persist for 15 days after the infection was detected at a clinic visit. We estimated that HIV-1 seroconversion would be detected 45 days after HIV-1 acquisition, under the assumption that infection would occur, on average, at the midpoint between visits and that visits occurred every 30 days. HIV-1 antibodies take 25 days to appear after infection [7], so antibodies would be detectable at a visit occurring 45 days after infection. Thus, we calculated that the total window of effect for *T. vaginalis* infection to influence HIV-1 susceptibility would be 60 days (15 + 45 days).

The multivariate model controlled for baseline educational level (≤8 vs. >8 years), parity (≤2 vs. >2), alcohol use, workplace (bar vs. nightclub), and vaginal washing practices and included time-dependent adjustment for genital tract infections other than with *T. vaginalis* (BV, vaginal candidiasis, genital ulcer disease, cervicitis, and gonorrhea), contraceptive method (no contraception or tubal ligation, oral contraceptive pills, depot medroxyprogesterone acetate [DMPA], Norplant, or intrauterine device [4]), age (<25, 25–29, 30–34, 35–39, and ≥40 years), duration of prostitution (<1, 2–4, 5–9, and ≥10 years), number of sex partners per week (≤1 vs. >1), sexual frequency per week (≤2 vs. >2), and condom use (<100% vs. 100%).

Continuous measures were dichotomized at the median from the enrollment visit. Similar to our modeling of trichomoniasis, we assumed an effect window of 60 days to capture the influence of other genital tract infections on HIV-1 susceptibility. For the sexual behavior variables (number of sex partners, sexual frequency, and condom use), an average was calculated for each year of follow-up, to capture average behavior over time.

We also performed univariate and multivariate analyses of risk factors for *T. vaginalis* acquisition, using Andersen-Gill proportional hazards models. Variables associated with incident *T. vaginalis* infection in univariate analysis ($P \leq .1$) were included in a multivariate model. As we have done previously [8], we assumed an effect window of 85 days to capture the effect of hormonal contraceptive use on *T. vaginalis* acquisition among women who changed contraceptive methods during follow-up (70 days of persistent effect of hormonal contraception after discontinuation of use +15 days from *T. vaginalis* acquisition to detection at a clinic visit, under the assumption of acquisition at the midpoint between monthly visits).

Results. A total of 1579 HIV-1–seronegative women were enrolled in the cohort, of whom 1335 (85%) returned for follow-up. Women who were lost to follow-up were generally similar to those included in the analyses, although they were slightly younger (median, 25 vs. 26 years; $P = .002$) and had a shorter duration of prostitution (median, 0.8 vs. 1 year, $P = .01$). Women who never returned for follow-up were not significantly different from those who returned in terms of number of sex partners per week, sexual frequency, condom use, contraceptive use, or baseline prevalence of *T. vaginalis* infection (data not shown).

Baseline characteristics of the 1335 women included in these analyses are provided in table 1. The median duration of follow-up was 566 days (interquartile range [IQR], 178–1330 days), and the median time between visits was 34 days (IQR, 28–54 days). A total of 3422 person-years of follow-up were accrued. There were 806 incident *T. vaginalis* infections (23.6/100 person-years), and 265 women seroconverted to HIV-1 (7.7/100 person-years).

In univariate analyses, infection with *T. vaginalis* was associated with a significantly increased risk of HIV-1 seroconversion (hazard ratio [HR], 1.60 [95% CI, 1.11–2.31]; $P = .01$).

Table 1. Enrollment characteristics ($n = 1335$).

Characteristic	Value
Age, median (IQR), years	26 (22–31)
Duration of prostitution, median (IQR), years	1 (<1–3)
Education, median (IQR), years	8 (6–10)
Parity, median (IQR)	2 (1–3)
Alcohol	1052 (79)
Bar worker ($n = 1262$)	929 (74)
Vaginal cleansing (any method)	1264 (95)
Water	303 (23)
Soap/other	961 (72)
Lubrication used for sex	295 (22)
Sex partners/week, median (IQR)	1 (1–2)
Sexual frequency/week, median (IQR)	2 (1–3)
Condom use, median (IQR)	100 (0–100)
100% condom use	836 (63)
Contraception	
Oral contraceptive pills	186 (14)
DMPA	267 (20)
IUD	28 (2)
Norplant	22 (2)
Genital tract infections	
Trichomoniasis	79 (6)
Genital ulcer disease	27 (2)
Neisseria gonorrhoeae	70 (5)
Cervicitis	206 (16)
Bacterial vaginosis	480 (37)
Vaginal candidiasis	177 (13)

NOTE. Data are no. (%) of subjects, unless otherwise specified. DMPA, depot medroxyprogesterone acetate; IQR, interquartile range; IUD, intrauterine device.

This association remained statistically significant after adjustment for sexual risk behaviors, STIs, and other potential confounding factors as detailed above (adjusted HR, 1.52 [95% CI, 1.04–2.24]; $P = .03$).

In an exploratory analysis evaluating risk factors for *T. vaginalis* infection, we found that shorter duration of prostitution, lower educational level, and use of alcohol were associated with a significantly higher risk of *T. vaginalis* infection (table 2). Acquisition of trichomoniasis was also more common among women who had concurrent cervicitis or concurrent BV. By contrast, 100% reported condom use and use of progesterone-only contraceptives (DMPA and Norplant) were associated with a lower risk of vaginal trichomoniasis. The results were similar in multivariate analyses.

Discussion. In the present prospective study, infection with *T. vaginalis* was associated with a significantly increased risk of HIV-1 acquisition. To our knowledge, this is the largest study to date to examine this relationship and is only the second study to show a statistically significant association. This is also one of only a few studies to investigate correlates of incident trichomoniasis in women.

Several lines of evidence suggest the biological plausibility of a causal association between vaginal trichomoniasis and increased risk of HIV-1 acquisition. First, infection with *T.*

Table 2. Correlates of incident infection with *Trichomonas vaginalis*.

Characteristic	Univariate		Multivariate	
	HR (95% CI)	P	aHR (95% CI)	P
Age				
<25 years	1.00		...	...
25–29 years	0.97 (0.73–1.29)	.9	...	...
30–34 years	1.08 (0.80–1.47)	.6	...	...
35–39 years	0.99 (0.70–1.40)	1.0	...	...
≥40 years	1.36 (0.90–2.05)	.2	...	...
Duration of prostitution				
≤1 year	1.00		1.00	
2–4 years	0.56 (0.42–0.75)	<.001	0.46 (0.35–0.62)	<.001
5–9 years	0.54 (0.39–0.76)	<.001	0.44 (0.31–0.62)	<.001
≥10 years	0.58 (0.38–0.88)	.01	0.41 (0.27–0.62)	<.001
Education ≤8 years	1.85 (1.46–2.36)	<.001	1.82 (1.41–2.35)	<.001
Parity >2	1.09 (0.86– 1.40)	.5		
Alcohol use	1.39 (1.05–1.85)	.02	1.32 (0.99–1.77)	.06
Bar worker[a]	1.26 (0.95–1.67)	.10	1.18 (0.90–1.53)	.2
Sex partners >1/week	1.07 (0.84–1.37)	.6	...	...
Sexual frequency >2/week	0.99 (0.78–1.27)	1.0	...	...
100% condom use	0.76 (0.63–0.92)	.004	0.85 (0.70–1.02)	.08
Contraception				
None	1.00		1.00	
OCP	0.76 (0.58–1.00)	.05	0.83 (0.63–1.10)	.2
DMPA	0.65 (0.43–0.97)	.04	0.69 (0.46–1.05)	.08
IUD	1.15 (0.53–2.49)	.7	1.42 (0.68–2.97)	.4
Norplant	0.11 (0.02–0.57)	.009	0.13 (0.03–0.61)	.009
Concurrent genital tract infection				
Genital ulcer disease	1.05 (0.54–2.02)	.9	...	...
Neisseria gonorrhoeae	1.37 (0.95–1.99)	.10	1.16 (0.77–1.73)	.5
Cervicitis[b]	1.56 (1.24–1.96)	<.001	1.60 (1.25–2.04)	<.001
Bacterial vaginosis	1.56 (1.31–1.87)	<.001	1.49 (1.24–1.79)	<.001
Vaginal candidiasis	0.85 (0.65–1.12)	.3		

NOTE. Risk estimates are based on Andersen-Gill proportional hazards analysis. aHR, adjusted hazard ratio; CI, confidence interval; DMPA, depot medroxyprogesterone acetate; HR, hazard ratio; IUD, intrauterine device; OCP, oral contraceptive pill.

[a] Bar work was associated with an increased risk of sexually transmitted infections and HIV acquisition in the Mombasa cohort [4].

[b] Cervicitis was defined by the presence of an average polymorphonuclear leukocyte count ≥30 cells/high-power field.

vaginalis leads to an inflammatory response with recruitment of CD4-bearing lymphocytes and macrophages to the vaginal and cervical mucosa [9, 10]. Second, punctate mucosal hemorrhages can occur in trichomoniasis, which potentially compromise the mechanical barrier to HIV-1 infection [11]. Third, *T. vaginalis* has been shown to degrade secretory leukocyte protease inhibitor, which can block HIV-1 attachment to cells [12]. Finally, trichomoniasis could increase the risk of HIV-1 acquisition by increasing susceptibility to BV or persistence of abnormal vaginal flora [13].

A recent meta-analysis that included all previously published prospective studies of the association between *T. vaginalis* and HIV-1 acquisition found a 1.5-fold (95% CI, 1.2–2.0-fold) increased risk of HIV-1 acquisition among women with vaginal trichomoniasis [2]. Our results, which used data from >3400 person-years of follow-up, are in agreement with these findings and thus provide the strongest evidence to date that infection with *T. vaginalis* may increase women's risk of HIV-1 acquisition by ∼50%.

Given the high prevalence of vaginal trichomoniasis globally, even a modest increase in the risk of HIV-1 acquisition from *T. vaginalis* infection could account for a high attributable risk for HIV-1 acquisition. One study recently estimated that 6.2% of HIV-1 infections among US women might be attributed to *T. vaginalis* [14]. The incidence of vaginal trichomoniasis is thought to be 4-fold higher in sub-Saharan Africa than in the United States [1], so this infection could be responsible for an even greater percentage of HIV-1 infections in African women.

Several features of the study's design support the validity of its findings. Prospective, monthly data collection allowed precise estimation of the timing of the exposure (*T. vaginalis* infection), the outcome (HIV-1 seroconversion), and potential confounding factors. The large sample size and long duration of follow-up allowed us to perform comprehensive multivariate analyses to adjust for potential confounding factors. The study was conducted in a population of women at high risk for both HIV-1 infection and STIs, making this an ideal setting in which both to identify this association and to consider possible intervention strategies.

The findings of the present study should be interpreted in the context of potential limitations. First, because *T. vaginalis* and HIV-1 share a common route of transmission, it is important to control for behaviors that simultaneously increase the risk for both infections. Accurate assessment of sexual risk can be difficult. We have previously shown that self-reported behavioral data from this population were associated with STIs, which may provide objective markers of sexual risk [15]. Nonetheless, there remains a potential for residual confounding. Second, if a causal association between trichomoniasis and HIV-1 acquisition exists, then the insensitivity of microscopy (compared with that of culture or of nucleic acid amplification

techniques) could lead to an underestimation of the true magnitude of the effect. Finally, some STIs (e.g., *Chlamydia trachomatis* and HSV-2 infections) were not tested or included in our analyses.

In this cohort of Mombasa FSWs, we identified recent entry into prostitution and lower educational level as correlates of incident *T. vaginalis* infection. These characteristics may be markers for other risk behaviors. We also found that *T. vaginalis* infection was associated with concurrent BV and cervicitis. Lower rates of trichomoniasis were seen among women using progesterone-only contraceptives, particularly Norplant. We have previously reported a significantly lower risk of trichomoniasis in women using DMPA [8]. The mechanism for a protective effect of progesterone-only contraceptives is not clear, but it has been suggested that exogenous hormones may interfere with binding to androgen and estrogen receptors that are present on *T. vaginalis* [16]. It will be important to determine whether this finding can be replicated in other settings.

Globally, there is a higher incidence of *T. vaginalis* infection than of any other curable STI [1]. Our finding associating this infection with increased risk of HIV-1 acquisition suggests that interventions targeted toward preventing vaginal trichomoniasis could reduce the spread of HIV-1. There is mounting evidence that other conditions that disrupt the normal vaginal milieu, including BV and vaginal candidiasis, also increase susceptibility to HIV-1 acquisition [4], but little attention has been given to the potential role of vaginal health as an HIV-1 prevention strategy. Interventions to prevent and treat trichomoniasis and to improve vaginal health in general could provide important female-controlled methods for reducing the risk of HIV-1 transmission to women.

References

1. World Health Organization (WHO). Global prevalence and incidence of selected curable sexually transmitted diseases: overview and estimates. Geneva: WHO, **1999**.
2. Rottingen JA, Cameron DW, Garnett GP. A systematic review of the epidemiologic interactions between classic sexually transmitted diseases and HIV: how much really is known? Sex Transm Dis **2001**; 28:579–97.
3. Laga M, Alary M, Nzila N, et al. Condom promotion, sexually transmitted diseases treatment, and declining incidence of HIV-1 infection in female Zairian sex workers. Lancet **1994**; 344:246–8.
4. Martin HL Jr, Nyange PM, Richarson BA, et al. Hormonal contraception, sexually transmitted diseases, and risk of heterosexual transmis-

sion of human immunodeficiency virus type 1. J Infect Dis **1998**; 178: 1053–9.

5. World Health Organization (WHO), Centers for Disease Control and Prevention (CDC). Guidelines for appropriate evaluations of HIV testing technologies in Africa. Harare, Zimbabwe: WHO, CDC, **2001**.

6. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. J Clin Microbiol **1991**; 29:297–301.

7. Busch MP, Lee LL, Satten GA, et al. Time course of detection of viral and serologic markers preceding human immunodeficiency virus type 1 seroconversion: implications for screening of blood and tissue donors. Transfusion **1995**; 35:91–7.

8. Baeten JM, Nyange PM, Richardson BA, et al. Hormonal contraception and risk of sexually transmitted disease acquisition: results from a prospective study. Am J Obstet Gynecol **2001**; 185:380–5.

9. Kiviat NB, Paavonen JA, Brockway J, et al. Cytologic manifestations of cervical and vaginal infections. I. Epithelial and inflammatory cellular changes. JAMA **1985**; 253:989–96.

10. Levine WC, Pope V, Bhoomkar A, et al. Increase in endocervical CD4 lymphocytes among women with nonulcerative sexually transmitted diseases. J Infect Dis **1998**; 177:167–74.

11. Fouts AC, Kraus SJ. *Trichomonas vaginalis:* reevaluation of its clinical presentation and laboratory diagnosis. J Infect Dis **1980**; 141:137–43.

12. Draper D, Donohoe W, Mortimer L, Heine RP. Cysteine proteases of *Trichomonas vaginalis* degrade secretory leukocyte protease inhibitor. J Infect Dis **1998**; 178:815–9.

13. Moodley P, Connolly C, Sturm AW. Interrelationships among human immunodeficiency virus type 1 infection, bacterial vaginosis, trichomoniasis, and the presence of yeasts. J Infect Dis **2002**; 185:69–73.

14. Chesson HW, Blandford JM, Pinkerton SD. Estimates of the annual number and cost of new HIV infections among women attributable to trichomoniasis in the United States. Sex Transm Dis **2004**; 31:547–51.

15. McClelland RS, Lavreys L, Katingima C, et al. Contribution of HIV-1 infection to acquisition of sexually transmitted disease: a 10-year prospective study. J Infect Dis **2005**; 191:333–8.

16. Ford LC, Hammill HA, DeLange RJ, et al. Determination of estrogen and androgen receptors in *Trichomonas vaginalis* and the effects of antihormones. Am J Obstet Gynecol **1987**; 156:1119–21.