

Prevalence of *Trichomonas vaginalis* infection among young reproductive age women in India: implications for treatment and prevention

Purnima Madhivanan^{A,B,E}, Melissa T. Bartman^C, Lauren Pasutti^C, Karl Krupp^A, Anjali Arun^{A,D}, Arthur L. Reingold^C and Jeffrey D. Klausner^B

^APublic Health Research Institute, 89/B, 2nd Cross, 2nd Main, Yadavgi, Mysore 570020, Karnataka, India.

^BSan Francisco Department of Public Health, 1360 Mission Street, Suite 401, San Francisco, CA 94103, USA.

^CUniversity of California Berkeley, School of Public Health, 104 Haviland Hall, Berkeley, CA 94720, USA.

^DVikram Hospital, No. 46, Vivekananda Road, Yadavgi, Mysore 570020, India.

^ECorresponding author. Email: purnima.madhivanan@sfdph.org

Abstract. **Background:** *Trichomonas vaginalis* infection is the most common curable sexually transmissible infection (STI) worldwide. The present study describes the burden and correlates of *T. vaginalis* infection among young reproductive age women in Mysore, India. **Methods:** Between November 2005 and March 2006, sexually active women aged 15–30 years were recruited from low-income peri-urban and rural neighbourhoods of Mysore, India. Participants were interviewed and offered a physical examination and testing for *T. vaginalis*, bacterial vaginosis, vaginal candidiasis, *Neisseria gonorrhoea* and herpes simplex virus type-2 antibodies. **Results:** Of the 898 participating women, 76 had a *T. vaginalis* infection (8.5%, 95% confidence interval [95% CI]: 6.7–10.5%). Nearly all (98%) participants were married and most reported their spouse as their main sex partner. The mean age at marriage was 16.9 years (s.d. 2.9 years) and two-thirds of the sample reported having first sexual intercourse before the age of 19 years. Risk factors independently associated with *T. vaginalis* infection included early age at first intercourse (adjusted odds ratio [OR] 2.09; 95% CI: 1.09–4.00), concurrent bacterial vaginosis (OR 8.21; 95% CI: 4.30–15.66), vaginal candidiasis (OR 2.40; 95% CI: 1.48–3.89) and herpes simplex virus type-2 infection (OR 3.44; 95% CI: 1.97–6.02). **Conclusion:** The burden of *T. vaginalis* infection at 8.5% is relatively high among a community sample of young reproductive aged women. Because this infection increases the risk of HIV transmission and is associated with adverse pregnancy outcomes, there is a need for increased screening and treatment of this easily curable sexually transmissible infection in India.

Additional keywords: correlates, epidemiology, sexually transmissible diseases, women.

Introduction

According to the World Health Organization, *Trichomonas vaginalis* is the most common curable sexually transmissible infection (STI) worldwide, with ~170 million to 190 million new cases each year.^{1,2} *T. vaginalis* infection is usually found concomitantly with other STIs, including chlamydia,³ gonorrhoea, syphilis,⁴ and herpes simplex virus type-2 (HSV-2) and is thought to be a sensitive marker of high-risk sexual behaviour.^{2,5–7} Recent research suggests that *T. vaginalis* infection may also be an important cofactor for HIV transmission and acquisition.^{8,9} In addition, *T. vaginalis* infection has been associated with adverse pregnancy outcomes such as premature rupture of membranes, preterm delivery and low birthweight.^{2,10–12}

Studies in India have shown the prevalence of *T. vaginalis* infection ranged from 1.2% to 28.5% across a variety of populations including obstetric and gynaecology clinic attendees,¹³ STI clinic attendees,¹⁴ commercial sex workers,¹⁵ and community-based populations.^{16–18} Previous studies estimated that 50–70% of *T. vaginalis* infection may be

asymptomatic, complicating treatment and prevention efforts.^{10,19} In addition, research suggests that higher number of lifetime sex partners, concurrent infection with other STIs, lower education, and older age are risk factors for *T. vaginalis* infection.

While *T. vaginalis* infection is considered an indicator for high-risk sexual behaviours, in India, the bulk of infections are among otherwise low risk populations. There is a dearth of data on the prevalence and risk factors for *T. vaginalis* infection among women in India. In the present paper we investigate the prevalence and epidemiological correlates of *T. vaginalis* infection among young married women in Mysore, India.

Methods

Study population

From November 2005 to March 2006, young sexually active non-pregnant women were recruited from low-income peri-urban and rural neighbourhoods of Mysore city using extensive community education and outreach. A detailed description of the recruitment process is described elsewhere.²⁰ In brief,

women were invited to visit the reproductive health clinics at Church of South India Holdsworth Memorial Hospital and Chitra's Hospital to participate in a prospective cohort study examining the relationship of vaginal infections and HSV-2 acquisition. To be included in the study, participants had to be between 15 and 30 years of age; reporting vaginal intercourse at least once in the previous 3 months; willing to undergo a pelvic examination; and planning to remain in the area for at least 6 months. The institutional review boards of the University of California, Berkeley, and Asha Kirana Hospital, Mysore, approved the study.

Data collection

All women provided signed informed consent at enrolment, and trained interviewers collected information in the following domains, using a standardised questionnaire in *Kannada* or *Urdu*.

- Sociodemographic variables included age, education, religion, marital status, monthly household income, occupation, and availability of a toilet at home.
- Reproductive and sexual health variables included past history (lifetime and prior 3 months) and current complaints of excess vaginal discharge, genital sores, burning or itching in the genitalia indicative of reproductive tract infections; contraceptive use; unprotected vaginal, oral and anal sex in prior 3 months and lifetime; number of sex partners in the past 3 months and lifetime; years with partner; condom use with partner (ever and last sex act) and lifetime drug and alcohol use.
- Partner characteristic variables include age, education, occupation, drug and alcohol use in lifetime and past 3 months, number of other sex partners in the past, having other concurrent sex partners, and travel away from home.

Specimen collection

A trained study clinician performed a pelvic examination to collect three vaginal swabs, two vaginal smears, and an endocervical swab. In addition, serum was collected for the detection of HSV-2 antibodies. Women were treated according to the US Centers for Disease Control and Prevention treatment guidelines.²¹ Women diagnosed with an STI were given additional medication for their partners.

Laboratory assessment

Diagnostic testing was completed in laboratories at Holdsworth Memorial Hospital and Vikram Hospital in Mysore. Saline wet-mount preparations of vaginal fluids were examined microscopically in the clinic for motile Trichomonads, clue cells, and yeast cells within 5 min of collection. Vaginal fluid specimens were cultured for *Trichomonas vaginalis* using InPouch TV culture kit, (Biomed Diagnostic, White City, OR, USA), and read daily for 5 days for the presence of trichomonads. Vaginal specimens were also cultured for *Candida* species (Biomed Diagnostic), and endocervical swabs were cultured for *Neisseria gonorrhoeae* in modified Thayer Martin medium (Biomed Diagnostic). Gram-stained vaginal smears were assessed for bacterial vaginosis (BV) by

two trained independent technicians using the Nugent score.²² Screening for HSV-2 antibodies was performed using an IgG type-specific enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's directions (Focus Technologies, Cypress, CA, USA). Testing for HIV, syphilis and chlamydia were not done due to funding limitations.

Data analysis

Data were analysed using Stata 9.0 (Stata Corporation, College Station, TX, USA). The prevalence of infection and 95% confidence intervals (CI) were estimated. Women were defined to be positive for *T. vaginalis* infection if they had positive wet-mount microscopy results and/or a positive *T. vaginalis* culture. They were defined to be negative for *T. vaginalis* infection if they had negative wet-mount microscopy results and a negative *T. vaginalis* culture. Descriptive analyses were conducted using Pearson χ^2 or Fisher's-exact test for categorical variables and *t*-test for continuous variables that are normally distributed.

The following variables were selected *a priori* to examine their association with *T. vaginalis* infection: sociodemographic variables such as the number of other people in household earning money, total monthly income, and amount of total household income provided by respondent were analysed as continuous variables; availability of toilet at home and alcohol use were analysed as dichotomous variables; and age, years of education, occupation and religion were examined as categorical variables. Reproductive and sexual health variables such as age at first sex, number of sex partners, tubectomy, having children, current complaints of abnormal vaginal discharge, genital itching, and burning with urination were analysed as binary variables. Condom use with the person's partner was analysed as a categorical variable as 'always', 'sometimes' and 'never'. Among partner characteristic variables, age and years of education were analysed as continuous variables; alcohol use as a dichotomous variable; and occupation and having other concurrent sex partners as categorical variables. Laboratory diagnosed infections included vaginal candidiasis, and HSV-2 infection were examined as binary variables and bacterial vaginosis was analysed as categorical variable as negative (0–3), intermediate (4–6), or positive (7–10) by Nugent Score.

Logistic regression was used to calculate the crude and adjusted odds ratios (OR) and corresponding 95% confidence intervals for the association between *T. vaginalis* infection and pre-selected variables. Risk factors found to be statistically significant at the 0.1 level were entered in a multivariable logistic regression model to further examine their association with *T. vaginalis* infection.

Results

Of the 996 eligible women who agreed to participate in the study, 898 completed all baseline procedures. Of those, 76 (8.5%, 95% CI: 6.7–10.5%) were diagnosed as having *T. vaginalis* infection. The median age of women in the study was 26 years (range: 16–30 years). Nearly all (98%) participants were married and most (878) reported their spouse as their main sex partner. Two-thirds of the sample reported having first sexual intercourse before the age of 19 years. Most of the

women had never used a condom (93.9%) or had used a condom sometimes (2.1%). Reversible family planning methods were uncommon, with a majority of women (63%) having had a tubal ligation after their last delivery. The sociodemographic characteristics of the study population are shown in Table 1.

Only 19 women reported having more than one sex partner in their lifetime. The same number of women also reported using condoms consistently, while the remaining reported never using them or using them inconsistently. Most (86%) participants had spouses who were older than themselves and who did not travel or spend time away from home. About 42% of the study participants said they did not know if their husband had other sex partners. Finally, over half of the women's husbands had less than 7 years of education.

Overall, 54% of the women had at least one laboratory-diagnosed reproductive tract infection with 8.5% having *T. vaginalis* infection (95% CI: 6.7–10.5%). There were five cases of *T. vaginalis* infections, diagnosed by saline wet-mount examination, that were negative on culture. Among women with culture-positive *T. vaginalis* infection, 41% were asymptomatic. Those who had symptoms most frequently reported abnormal vaginal discharge (37%), genital itching (18%), and a burning sensation in the genitalia (16%).

Women with *T. vaginalis* infection were more likely to be older, non-Muslim, having lower levels of education, having undergone tubal ligation, reporting more than one sex partner and married to a partner with no education as compared with women without *T. vaginalis* infection. There was no significant difference in the prevalence of *T. vaginalis* infection among women complaining of abnormal vaginal discharge as compared with women with no complaints of abnormal vaginal discharge ($P=0.24$). In addition, women with *T. vaginalis* infection were more likely to have concurrent BV (19.4% v. 2.7%; $P<0.0001$), vaginal candidiasis (13% v. 6.4%; $P<0.001$) or HSV-2 antibodies (22% v. 6.8%; $P<0.0001$) as compared with those without *T. vaginalis* infection.

Unadjusted analysis showed the odds of *T. vaginalis* infection were 0.35 (95% CI: 0.18, 0.70) for Muslim women as compared with Hindu women, and 0.48 (95% CI: 0.29, 0.80) for women with a toilet at home as compared with those without. The odds for *T. vaginalis* infection increased to 3.79 for women who had more than one sex partner in their lifetime as compared with women with only one partner. The odds also increased for women who had a tubal ligation (1.71; 95% CI: 1.01, 2.91), and whose age at first intercourse was less than 15 years (2.12; 95% CI: 1.30, 3.67). The odds of *T. vaginalis* infection were 3.8 (95% CI: 2.24, 6.7) among women who had HSV-2 antibodies as compared with women without HSV-2 antibodies. The odds of *T. vaginalis* infection were 8.8 (95% CI: 4.65, 16.8) among women who were diagnosed with BV and 8.1 (95% CI: 4.12, 16.9) among women with an intermediate stage of BV as compared with women who were negative for BV. Similarly, the odds for *T. vaginalis* infection were 2.2 (95% CI: 1.35, 3.49) for women with vaginal candidiasis as compared with women without candidiasis.

Factors that were significant in the unadjusted analysis were included in a multivariable model to examine for confounding. Those that remained independently associated with increased relative odds of *T. vaginalis* infection included diagnosis of

Table 1. Sociodemographic characteristics association with *Trichomonas vaginalis* infections among 898 young reproductive age women in Mysore, India
n.s., not significant

Characteristic	Total		<i>T. vaginalis</i> infection		P-value
	N	%	N	%	
Age categories					0.001
15–20 years	57	6.4	5	8.7	
21–25 years	367	40.9	13	3.5	
26–30 years	474	52.8	58	12.2	
Years of education					0.09
0	240	26.7	28	11.7	
1–7	272	30.3	22	8.1	
>7	386	43.0	26	6.7	
Religion					0.005
Hindu	621	69.2	63	10.1	
Muslim	258	28.7	10	3.9	
Christian	19	2.1	3	15.8	
Occupation					0.9
Housewife	673	74.9	56	8.3	
Unskilled	175	19.5	15	8.6	
Skilled	50	5.6	5	10.0	
Occupation of the husband					0.43
Unskilled	391	43.5	31	7.9	
Skilled	406	45.2	39	9.6	
Skilled-drivers	101	11.3	6	5.9	
Have toilet at home					0.004
Yes	391	43.5	39	9.9	
No	507	56.5	37	7.3	
Underwent tubal ligation					0.04
Yes	566	63.0	56	9.9	
No	332	37.0	20	6.0	
Age at first sex					0.04
<15 years	167	18.6	24	14.4	
15–16 years	268	29.8	18	6.7	
17–18 years	252	28.1	20	7.9	
19–20 years	119	13.3	7	5.9	
≥21 years	92	10.2	7	7.6	
Condom use					0.75
Never	843	93.9	71	8.4	
Sometimes	36	4.0	4	11.1	
Always	19	2.1	1	5.3	
Number of sex partners					0.007
One	878	97.9	71	8.1	
More than one	19	2.1	5	25.0	
Herpes simplex virus-2 antibodies ^A					0.0001
Present	100	11.3	22	2.2	
Absent	782	88.7	53	6.8	
Bacterial vaginosis ^A					0.0001
Positive (n.s. 7–10)	165	19.1	32	19.4	
Intermediate (n.s. 4–6)	133	15.4	24	18.1	
Negative (n.s. 0–3)	566	65.5	15	2.7	
Vaginal candidiasis					0.001
Present	277	30.9	36	13.0	
Absent	621	69.2	40	6.4	

^ADenominators differ because of missing data.

concurrent BV (adjOR 8.21; 95% CI: 4.30, 15.66) or vaginal candidiasis (adjOR 2.40; 95% CI: 1.48, 3.89); presence of HSV-2 antibodies (adjOR 3.44; 95% CI: 1.97, 6.02) and lower age at first intercourse (adjOR 2.09; 95% CI: 1.09, 4.00) (Table 2).

Table 2. Select sociodemographic, reproductive health and laboratory diagnosed infections associated with *Trichomonas vaginalis* infection among young reproductive age women in Mysore, India
CI, confidence interval; OR, odds ratio

Characteristic	<i>T. vaginalis</i> infection		Adjusted OR	95% CI
	Unadjusted OR	95% CI		
Age categories				
15–20 years	Ref ^A			
21–25 years	0.38	0.13, 1.11		
26–30 years	1.45	0.56, 3.78		
Education (in years)				
0	Ref		Ref	
1–7	0.67	0.37, 1.19	0.86	0.42, 1.76
>7	0.55	0.31, 0.96	0.69	0.33, 1.45
Religion				
Hindu	Ref		Ref	
Muslim	0.35	0.18, 0.70	0.87	0.39, 1.91
Christian	1.66	0.47, 5.85	1.63	0.36, 7.29
Own toilet				
Yes	0.48	0.29, 0.80	0.60	0.32, 1.17
No	Ref		Ref	
Underwent tubal ligation				
Yes	1.71	1.01, 2.92	1.61	0.85, 3.07
No	Ref		Ref	
Age at first sex				
<15 years	2.12	1.30, 3.67	2.09	1.09, 4.00
≥15 years	Ref		Ref	
Number of sex partners				
One	Ref		Ref	
More than one	2.98	0.96, 9.24	2.54	0.51, 12.85
HSV-2 antibodies				
Present	3.88	2.24, 6.72	3.08	1.56, 6.11
Absent	Ref		Ref	
Bacterial vaginosis				
Positive	8.84	4.65, 16.79	7.57	3.78, 15.14
Intermediate	8.09	4.12, 15.92	8.85	4.29, 18.26
Negative	Ref		Ref	
Vaginal candidiasis				
Present	2.17	1.35, 3.49	2.34	1.34, 4.10
Absent	Ref		Ref	

^ALogistic regression assigned odds ratio (OR) = 1.00 for the referent category for each variable.

Discussion

In this large sample of young married women, the prevalence of *T. vaginalis* infection at 8.5% was relatively high compared with studies among similar populations in India.^{16,17} Consistent with the literature, almost half of the infections were asymptomatic, posing a major challenge for control of an infection shown to be associated with adverse pregnancy outcomes and acquisition of HIV infection.^{2,7,12}

In the present study, certain variables associated with *T. vaginalis* infection in unadjusted analyses were similar to those found in other studies. There was an increased odds of infection with lesser years of education and the lack of a toilet, likely proxies for lower socioeconomic status and poor access to medical care.^{23,24} Additionally, as Kaestle and colleagues have observed, younger age at first intercourse was associated with higher odds of *T. vaginalis* infection as compared with older age

but the effect diminished with increasing age.²⁵ Finally as reported in many studies, there were increased odds of *T. vaginalis* infection in women co-infected with other reproductive tract infections.^{23,26–29} Unlike other studies, however, data from the present study showed that women who had undergone tubal ligation had increased odds of infection as compared with women without tubal ligation and Muslim women had reduced odds of infection as compared with Hindu women, but both of these effects disappeared in multivariable analysis. Furthermore, the present study did not find any association of *T. vaginalis* infection with several previously reported risk factors such as partner characteristics including education, occupation, drug and alcohol use, and other sex partners; women's report of daily alcohol consumption and infertility.^{23,30,31}

In multivariable analysis, risk factors for *T. vaginalis* infection included lower age at first intercourse, HSV-2 seropositivity, and the presence of concurrent BV or vaginal candidiasis. Early age at first intercourse may simply be a proxy for cumulative sexual exposure³² and high co-infection rates with other reproductive tract infections (RTI)/STIs raises the possibility that *T. vaginalis* infection may either increase susceptibility for, or share common pathways with, these other infections.^{33,34} It is worth noting that in the present study, women with 'intermediate' stage BV by Nugent score had similar odds for *T. vaginalis* infection compared with women 'positive' for BV. This is an important observation because women with 'intermediate' stage BV have traditionally been ignored in treatment guidelines.

Our findings should be interpreted in light of the following limitations. First, because our study sample was limited to women who came from the communities where outreach and education programs were conducted, findings may not be generalisable to other populations. Second, self-reported sexual behaviours may have been affected by response bias, social desirability bias and poor recall. This is particularly true with regard to the reported number of sex partners and sexual risk behaviours among Indian women in healthcare settings where there is often a perceived lack of confidentiality, anonymity, or privacy. In spite of careful attention to gender matching of interviewers with participants and provision of private space for interviews, we believe it is likely that there may be under-reporting of sexual risk behaviours in our sample. Further research is warranted to assess the validity of self-reported behaviours among women in India. Third, due to the cross-sectional nature of this analysis, we are unable to analyse the temporality of the associations between *T. vaginalis* infection and other RTIs. Because there were no cases of gonorrhoea detected and we did not test for chlamydia in our sample, we are unable to examine the relationship of *T. vaginalis* with these two infections. Additionally, because all participants in the study were married, we were unable to examine the relationship of *T. vaginalis* infection and marital status. Finally, because partners were not interviewed, partner characteristics including risk behaviours were gathered from the women themselves, leading to a potential for misclassification.

Despite these limitations, our study among a large population of young married women showed a relatively high prevalence of this treatable STI. Based on this and other community

samples,^{17,35} there may be as many as 14–22 million *T. vaginalis* infections among reproductive age women and 6–10 million of these infections may be asymptomatic. This takes on added importance in a setting like India, which has as many as 3–4 million preterm deliveries annually³⁶ and an increasingly feminised HIV epidemic³⁷ – two important health outcomes associated with *T. vaginalis* infection.

Given the public health implications of *T. vaginalis* infection in the mainstream reproductive age population, there is a need to rethink current public health policy on this easily treatable STI. Existing strategies focus on high-risk populations, ignoring the bulk of the disease burden in India. Furthermore, treatment guidelines use syndromic management of RTI/STI, an ineffective approach when almost half of *T. vaginalis* infections are asymptomatic and there is no provision for partner treatment. With increasing availability of simple and inexpensive point-of-care tests for *T. vaginalis* infection, there is a growing need for further evaluation and implementation of point-of-care screening particularly in settings where young women seek healthcare.

Funding

The Fogarty AIDS International Training and Research Program (Grant 1-D43-TW00003–16) supported the study but had no role in conducting the study or manuscript preparation. Focus Technologies (Cypress, CA, USA) donated HSV-2 ELISA test kits, BioMed Diagnostics (White City, OR, USA) donated TV, NG, and candida growth medium for the study, and Cipla Limited (Mumbai, India) donated oral Acyclovir. None of the companies provided monetary funding or had any role in the study.

Conflicts of interest

JDK has received funding for conducting research from Focus Technologies. All other authors declare no conflicts of interest.

Acknowledgements

For their generous assistance on this project, the authors would like to thank the following people and organisations: Directors of Asha Kirana Hospital (Mothi Sarvode), Chitra's Hospital (Mahesh Kumar), Church of South India Holdsworth Memorial Hospital (SC Karat); Dr Chitra Karat, Dr Varalakshmi Chandrasekaran and all the non-government organisations who assisted with outreach programs; Focus Technologies, Biomed Diagnostics and Cipla Limited for their generous donations; and the study individuals for their participation.

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Manuscript received 6 April 2009, accepted 31 July 2009