Trichomonas vaginalis: underdiagnosis in urban Australia could facilitate re-emergence

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ABSTRACT

Objectives *Trichomonas vaginalis* (TV) has a low profile in urban sexually transmitted infection (STI) clinics in many developed countries. The objective of this study was to determine the true prevalence of TV in an Australian urban sexual health setting using sensitive molecular diagnostic techniques.

Methods A cross-sectional study investigating the aetiology of cervicitis in women attending two urban sexual health clinics in Sydney, Australia, enrolled 356 consecutive eligible women from 2006 to 2008. The diagnostic yield from the standard clinical practice of discretionary high vaginal wet preparation microscopy in women with suspicious vaginal discharge was compared with universal use of nested PCR for TV of cervical samples.

Results TV was detected by PCR in 17/356 women (4.8%, 95% CI 2.8 to 7.5%), whereas only four cases (1.1%, 95% CI 0.3 to 2.8%) were detected by discretionary wet preparation microscopy. Eleven of the 17 women (p=0.003) were of culturally and linguistically diverse background. Additionally, cervicitis was found to be significantly associated with TV, RR 1.66 (1.14 to 2.42), p=0.034.

Conclusions Traditional TV-detection methods underestimate TV prevalence in urban Australia. The TV prevalence of 4.8% by PCR testing in this study exceeds previously reported urban Australian TV rates of <1%. An increase in trichomoniasis-associated adverse reproductive outcomes and enhanced HIV transmission poses a salient public health threat. Accordingly, TV warrants a higher profile in urban STI clinic settings in developed countries, and we suggest that priority be given to development of standardised molecular TV detection techniques and that these become part of routine STI testing.

INTRODUCTION

Trichomonas vaginalis (TV) is a sexually transmitted infection (STI) causing significant morbidity worldwide. Trichomonads are highly site-specific protozoan parasites. In women, TV infects the lower urogenital tract, causing superficial vaginal and cervical ulceration. Typical symptoms include frothy yellow discharge, itch, odour, dyspareunia and occasionally vaginal bleeding. Infection of the urethra and paraurethral glands causes dysuria and frequency.[1] However, at least one-third of infected women may be asymptomatic.[2] Trichomoniasis has been associated with premature rupture of membranes,[3] pelvic inflammatory disease (PID),[4] cervicitis[5 6] and enhanced risk of HIV transmission.[7] Men may present with balanitis, urethral discharge or dysuria, but again, high rates of asymptomatic carriage have been reported. A recent US STI clinic study detected TV in 72% of male partners of infected females, and of these men, 77% were asymptomatic.[8] The duration of TV infection in women may be prolonged for up to 3–5 years but only about 4 months in men.[9] The natural history of TV infection is not well defined.

Trichomoniasis is the most common curable STI. In 1999, WHO estimated 174 million new cases per year, more than double the number of *Chlamydia trachomatis* cases and treble the cases of gonorrhoea. The high TV prevalence worldwide is concentrated in developing countries and socio-economically disadvantaged groups, with a dramatic decline in TV rates in some developed countries in the past few decades.[10] TV prevalence in urban Australia is reportedly low based upon routine wet preparation diagnostic methods.[9 10] Australian rates of TV peaked in the 1950s at 20–30% and rapidly declined through the 1960s and 1970s to below 1% in 1990.[10] This has been attributed to the combination of widespread use of the Nitroimidazoles, and increased surveillance through Papanicolaou smears.[9 10] This decline and the fact that TV is not a notifiable disease in Australia have led to the present situation where testing for TV has assumed a very low priority in urban Australian settings. An audit of commercial sex workers (CSW) undergoing regular STI screening at a sexual health clinic in Melbourne in 2003 reported a very low incidence of TV at 0.11 per 100 person months.[11] By contrast, TV prevalence in indigenous Australian women, in remote Northern Territory, was high at 25% in a self-sampling PCR-method-based study.[12] The prevalence of TV by PCR among US women of reproductive age was recently found to be 3.1%, with an even lower prevalence of 1.3% in the subgroup of non-Hispanic white women.[13]

We are conducting a prospective study investigating the prevalence and aetiology of cervicitis using molecular diagnostic techniques in women attending two urban STI clinics in Sydney, Australia. In this paper, we report the prevalence of TV by PCR testing against traditional methods of detection, and compare our findings with previous data from other Australian urban STI clinics.

METHODS

This study was conducted in two urban Sydney STI clinics from July 2006 to December 2008. Ethics approval was granted by the South Eastern

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Sydney and Illawarra Area Health Service Human Research Ethics Committee and the Sydney South West Area Heath Service Ethics Review Committee (Royal Prince Alfred Hospital Zone).

Subject selection

Women were eligible for this study if they were 18 years or older, had been sexually active in the previous 3 months and required an internal examination. The parent cervicitis study protocol excluded women if they had been previously enrolled, had clinical PID, had received antibiotics or undergone gynaecological intervention in the previous month, had an intrauterine contraceptive device, were currently menstruating or pregnant, attending for sexual assault or unsuitable for enrolment due to psychosocial ill health or comprehension difficulties. Of a total of 957 consecutive first attendances, 561 women (58.6%) were ineligible, 40 (4.2%) declined, and 356 (37.2%) were enrolled. The most common reasons for ineligibility included examination not required (30.7%), antibiotics in previous month (14.2%), pregnancy (8.6%) and no sex in previous 3 months (8.3%). Women with clinical PID comprised 3.1% of ineligible women. This report is an interim analysis of 356 consecutive first attendances of women enrolled in the parent cervicitis study.

Sampling procedure

A sterile speculum was used to visualise the vagina and cervix. A Gram stain was performed on all cervical and high vaginal swabs (HVS). Due to the nature of the parent study, HVS for wet preparation microscopy was discretionary based upon clinical suspicion of TV infection, in keeping with usual clinical practice, and so not performed for all women. The endocervix was sampled in all women, with a sterile cotton swab (Copan, Brescia, Italy) for Gram stain and bacterial agents. An additional endocervical sterile cotton swab was taken and placed in viral transport medium 199 (GIBCO Invitrogen, Grand Island, New York) and stored at −70°C for subsequent PCR testing for TV by the method described below. Swabs from all women were tested for TV by PCR.

Cervicitis was defined as >30 polymorphonuclear cells per high-powered field (PMN/hpf) in at least three non-adjacent fields of cervical mucus on Gram stain of the first endocervical swab. Bacterial vaginosis (BV) was defined by Nugent score from Gram stain of the HVS.

Nucleic acid extraction and PCR amplification

Swabs were suspended in 500 µl of viral transport medium (above) before extraction of the total nucleic acid using a robotic extraction machine (MagNaPure LC, Roche, Germany) applying the Total NA protocol according to the manufacturer's instructions (Roche, Germany). Extracts were stored at 4°C before testing within 48 h of collection.

Detection of TV was performed using a nested PCR. Briefly, the first-round reaction comprised 10 µl of template in a 50 µl of PCR reaction mixture containing 9 µl of nuclease-free water, 25 µl of 2× iScript reaction mix (BioRad, Sydney, Australia), 0.5 µM each primer: TricV-OF (5′ CTATTGTCGAACATTGGTCTTACCCTC 3′) and TricV-OR (5′ TCTGTGCCGTCTTCAAGTATGCCC 3′)[14 15] and 1 µl of iScript RT enzyme (BioRad). (This commercial master mix is used in our laboratory for the amplification of both RNA and DNA templates). Cycling conditions were 50°C for 30 min; denaturation at 95°C for 15 min, then 35 cycles of 94°C for 45 s, 57°C for 45 s and 72°C for 1 min; a final extension of 7 min at 72°C; and a 4°C hold. A second round reaction comprised 2 µl of first-round product included in a 50 µl reaction mixture containing 18 µl of nuclease-free water, 25 µl of Amplitaq gold PCR Master Mix (Applied Biosystems, Foster City, California, USA) and 0.5 µM of each primer: TricV-IF (5′ CTCAGTTCGCAAAGGCAGTCCTTGA 3′) and TricV-IR (5′ GCTTGGAGAGGACATGAACTTCGGA 3′).[14] Cycling conditions included denaturation and activation at 95°C for 5 min, 33 cycles of: 94°C for 20 s, 57°C for 20 s, 72°C for 20 s; a final extension at 72°C for 10 min and a 4°C hold. PCR products of 206 bp were expected for TV-positives and were visualised by gel electrophoresis. Using positive controls from either culture-proven or molecularly proven sources, sensitivity was assessed by measuring the limit of detection (10^2 copies per reaction) of plasmid constructs of the target sites as previously described[14] and is estimated to be 95–98%. The specificity was determined as follows: confirmation by probe hybridisation following the PCR amplification, and DNA sequencing performed on PCR products of the first 10 TV positive samples from the study. Nucleotide BLAST on NCBI site (for all 10 samples) confirmed that all DNA sequences produced from sequencing were TV. The results from the above methods confirmed that the assay has 100% specificity.

Analysis

We report the prevalence with 95% CI of TV by traditional methods and PCR methods. Population characteristics of women with and without TV were compared using χ^2 testing. p Values <0.05 were considered statistically significant. Data were analysed with SAS software SAS Institute (Cary, North Carolina).

RESULTS

Prevalence of TV by PCR testing was 17/356 (4.8%, 95% CI 2.8 to 7.5%). Clinical suspicion prompting discretionary wet-mount preparation microscopy identified TV in only 4/356 women (1.1%, 95% CI 0.3 to 2.8%). PCR identified a higher percentage of women with TV (p=0.0003).

Of the 17 women positive for TV by PCR testing, only 11 had wet-preparation microscopy performed. The use of discretionary wet preparation was not significantly different in women with and without TV by PCR (p=0.498). Detection by Papanicolaou (Pap) smear occurred in only two of the 11 Pap smears done in women with TV. The mean age of the women with TV, 33.2 years, was not significantly different from the mean age of women without TV, 30.7 years (p=0.221) (table 1). Significantly, 11/17 women with TV (p=0.003) were of culturally and linguistically diverse background and identified consorts from populations of higher TV prevalence overseas (Africa, China, Sri Lanka, South America, Lebanon, Black American). Three women identified rural or 'bush' Australian contacts. Five women were CSW. No cases of TV were indigenous women. Six cases had BV, and three had concurrent STIs (two with *Chlamydia* and one with HIV and active genital herpes). Dysuria was significantly associated with women with TV (p=0.014). Prevalence of cervicitis in women without TV was 39%, compared with 65% (11/17) in women with TV, giving a RR of cervicitis in the presence of TV of 1.66 (95% CI 1.14 to 2.42) p=0.034.

DISCUSSION

These data show that traditional methods of detection greatly underestimate the prevalence of TV. We report a TV prevalence of 4.8% by PCR testing, whereas clinical suspicion prompting discretionary wet-preparation microscopy identified TV in only 4/356 women (1.1%) in the same study population. The lower

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Table 1 Characteristics of women with and without *Trichomonas vaginalis* (TV) by PCR

Characteristic	Women with TV n=17	Women without TV N=339	p Value*
Mean age (years)	33.4	30.7	0.221
Culturally and linguistically diverse†	11 (65%)	104 (31%)	0.003
Condoms always	4 (24%)	75 (22%)	0.892
>1 partner last 3 months	6 (35%)	95 (28%)	0.516
Commercial sex workers	5 (29%)	58 (17%)	0.195
Concomitant STI‡	2 (12%)	24 (7%)	0.469
Signs and symptoms			
Dysuria	7 (41%)	59 (17%)	0.014
Vaginal discharge	11 (65%)	143 (42%)	0.067
Bacterial vaginosis§	6 (35%)	79 (23%)	0.258

*p Values <0.05 are statistically significant.
†Defined as women identifying at clinic registration as preferring a language other than English, speaking a language other than English at home, or identifying a non-English ethnic background.
‡Concomitant chlamydia or gonorrhoea.
§Defined by Nugent score on Gram stain of high vaginal swab.

Key messages

- We report a higher than previously reported prevalence of *Trichomonas vaginalis* (TV) of 4.8% by PCR methods in an urban Australian sexually transmitted infection (STI) clinic setting.
- TV is likely underdiagnosed in urban STI clinic settings using only traditional methods of detection. This may presage re-emergence with important Public Health consequences.
- We find TV is significantly associated with women of culturally and linguistically diverse backgrounds, and cervicitis is significantly associated with TV (RR 1.66, p=0.034).
- TV warrants a higher profile. Priority should be given to the development of standardised molecular TV detection techniques for inclusion in routine STI testing.

case-detection rate by discretionary use of wet-preparation microscopy (and Pap smear) observed here reflects the inadequacy of relying solely upon these methods for detecting TV. Wet-mount microscopy has a sensitivity as low as 52%,[16] depending on specimen handling, skill of the microscopist and TV organism load in specimen. Additionally, wet-preparation microscopy is not routinely performed, even in many STI clinic settings and laboratories.[8 17]

These TV prevalence rates by PCR are higher than previously reported rates of <1% in other urban Australian STI clinic studies, sampling CSWs using traditional diagnostic methods.[10 11] This difference could signal a true rise in TV prevalence or be attributed to the more sensitive PCR diagnostic techniques employed here as well as sampling a less clinically homogeneous, highly screened population than those represented in previous literature.[9 11] However, due to the relatively low population prevalence of TV, the positive predictive value of these PCR findings may be reduced. On the other hand, these PCR data may potentially underestimate the true prevalence due to the strict inclusion criteria of the parent study protocol and retrospective PCR testing of stored, frozen and thawed specimens. Cervical specimens were used for this study, but the performance of cervical and vaginal specimens for TV PCR testing has been shown to be comparable.[18]

An important finding in our study was the high proportion (11/17, p=0.003) of women with TV who were of culturally and linguistically diverse backgrounds. Urban society in Australia, as in many other industrialised countries, is very culturally diverse with a highly mobile population. Accordingly, sexual networks, particularly of migrant and first-generation Australians often include consorts who come from or travel to populations with higher TV prevalence. We found the symptom of dysuria to be significantly associated with women with TV, highlighting the need for clinicians to consider the possibility of TV especially in the absence of other causes of dysuria. We did not find any association between TV and BV, although others have.[8] BV may mask the presence of TV. Standard BV treatment with nitroimidazoles will effectively eradicate coexistent TV infection, but partners of women with concomitant BV and TV will remain untreated, potentially facilitating the persistence and spread of TV.

Cervicitis was associated with TV (RR 1.66, p=0.034) supporting the role of TV in the aetiology of cervicitis. Non-gonococcal, non-chlamydial cervicitis or non-specific cervicitis comprises a major proportion of reported cervicitis,[5 6] and TV has been recently recognised as an aetiological agent in this condition.[5] Molecular testing for TV in STI populations is likely to shed further light on the aetiology of non-specific cervicitis in women, a current area of research need.

Coinfection with another STI, including TV, remains a significant risk factor for HIV acquisition.[7] Women increasingly carry the burden of the HIV epidemic in many developing and westernised countries.[19] As many screening programmes are targeting younger women, older groups of sexually active women where TV predominates[9] may be overlooked, potentially contributing to the TV reservoir in the community. The recent rise in gonorrhoea in women in some industrialised nations and noted in our own region[20] may indicate relaxation of safer sex practices, possibly presaging a rise in STIs with re-establishment in these sexually active populations and increased opportunity for HIV transmission.

If not specifically sought, TV will not be detected. Presently sensitive, standardised detection methods for TV are seldom commercially available, relying on in-house laboratory molecular methods. This, coupled with the low profile of TV, has led to infrequent uptake of *any* testing for TV in many clinical settings. Our findings suggest that when sensitive diagnostic tests are routinely or selectively applied, TV may be found to be more prevalent than previously thought. These concerns echo those of others[2 7–9 16 21] that priority be given to developing standardised, sensitive, cost-effective techniques for TV detection.

In summary, we report a higher than previously reported prevalence of TV, by PCR testing, in an urban Australian STI clinic population. We suggest that the current low profile of TV and the variable application of insensitive tests for case detection have led to underdiagnosis of TV. In turn, this could facilitate a re-emergence of TV, particularly in culturally diverse urban populations. Trichomoniasis has the potential to impact significantly on reproductive health of women and increase HIV transmission. Accordingly, TV warrants a higher profile in urban settings in developed countries. With this demonstration of higher than expected TV prevalence, a call is justified for the development and application of standardised PCR TV-detection techniques to facilitate case-finding, surveillance and continued control of this STI.

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Competing interests None.

Patient consent Obtained.

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Contributors MJL and PK: designed and implemented the clinical research project, collected clinical samples and clinical data and supervised designated clinicians in sample and data collection, undertook data entry and statistical analysis and wrote most of the manuscript (excluding lab methods section). ZN, NR, B Rayner, CJM and WDR: assisted with design of the study, processing and storage of samples and undertook the PCR testing of the clinical samples and contributed to writing the manuscript (lab methods). RGC: assisted with the design of the study, choice of statistical methods, and contributed to reviewing the manuscript. KM: Assisted with the database management and statistical analysis and reviewed manuscript.

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