

Original Article

Prevalence of *Trichomonas vaginalis* in women of reproductive age at a family health clinic

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Abstract

Introduction: *Trichomonas vaginalis* is considered the most prevalent curable sexually transmitted infection, and its occurrence exceeds that of gonococcal and chlamydia infections. This parasite has been identified as responsible for the increased risk of transmission of HIV and has also been associated with prostate and cervical cancer. Many carriers of *T. vaginalis* are asymptomatic and, when experiencing a health problem, they most often have nonspecific symptoms. The aim of this research was to estimate the presence of *T. vaginalis* and the associated factors in women of childbearing age at a primary health care clinic in the Federal District of Brazil.

Methodology: A cross-sectional study was conducted with consecutive sampling of an outpatient population of women of childbearing age (excluding minors and pregnant women). The women answered a questionnaire and were examined. After vaginal pH measurement and whiff testing, a vaginal secretion sample was obtained for inoculation in TYM, a specific *T. vaginalis* culture medium.

Results: The presence of *T. vaginalis* was identified in 16% of the sample. Fewer lifetime sexual partners and consistent condom use were identified as factors of protection against the infection. Complaints of dyspareunia were proportionally higher among women with positive cultures for *T. vaginalis*.

Conclusions: The prevalence of *T. vaginalis* infection was high in the sample studied. The infection was positively associated with the number of lifetime sexual partners, and consistent condom use was a protective factor. Vaginal complaints were more common among women with *T. vaginalis*, but only dyspareunia had significant association.

Key words: genital infections; sexually transmitted diseases; *Trichomonas vaginalis*; primary health care.

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Introduction

Trichomonas vaginalis is a sexually transmitted flagellated protozoan extracellular parasite of humans [1,2]. It is estimated to be the most common curable sexually transmitted infection (STI) in the world [3].

T. vaginalis infection can impair the reproductive success of men and women since it is associated with the occurrence of pelvic inflammatory disease [4,5] and can reduce sperm viability [6]. The parasite is also associated with premature rupture of membranes, preterm labor, and low birth weight [7,8]. Studies have also reported an association with increased risk of HIV transmission [9], prostate cancer [10], and cervical neoplasia [11].

It is estimated that the occurrence of *T. vaginalis* is twice the estimated incidence of infections caused by *Chlamydia trachomatis* [12], and it may be considered the most prevalent curable sexually transmitted infection [3,13]. In contrast to other STIs, *T. vaginalis*

more frequently affects people in older age groups [14,15].

T. vaginalis can cause asymptomatic or oligosymptomatic infections, or lush vulvovaginitis, with intense discomfort [13]. Commonly reported symptoms include vulvar irritation, yellow-greenish vaginal discharge, and unpleasant odor [16].

The prevalence of *T. vaginalis* in each age group depends on several factors, including the technique used to assess this prevalence [17-20]. For example, research using culture, wet mount, and Pap smear found a prevalence of 2.6% in women attending primary health clinics in Uberlândia, MG, in the southeast region of Brazil [18]. The prevalence was highest among women 40 years of age or older. This finding was consistent with that reported by other authors [21]. A survey that included more than 140,000 women in the Federal District, Midwest region of Brazil, found a prevalence of 7.3% [22].

Although there are different diagnostic techniques to detect the presence of the parasite, the diagnosis is often only presumptive, based on complaints registered by women. *T. vaginalis* infection increases the risk of HIV transmission and acquisition [9,23-29], and this protozoan is a suspected potential vector for other pathogens, since *T. vaginalis* can harbor *Mycoplasma hominis* [30,31]. Furthermore, some *T. vaginalis* strains are infected by a dsRNA virus from the genus *Trichomonasvirus*, named TVV [32]. The presence of TVV and *M. hominis* may affect the pathogenicity of the parasite [33,34].

Various techniques have been used to diagnose the infection, including direct microscopy and cultures of vaginal secretion. Additionally, recent studies report the use of molecular techniques [18,35] and serology [36].

The Brazilian Federal District Health Department has no established routine for the laboratory diagnosis of *T. vaginalis*. Cases are diagnosed clinically, using syndromic management, or even by the occasional finding of the parasite in a Pap smear. Although the Pap smear has specificity for *T. vaginalis*, there are sensitivity limitations that prevent the use of this technique for the diagnosis of infection [37]. The parasite has also been identified, occasionally, in urine samples.

The syndromic approach to sexually transmitted diseases involves a diagnostic and treatment strategy recommended by the World Health Organization [38] and adopted by the Brazilian Ministry of Health. This approach aims to increase the sensitivity of the identification of these diseases and the consequent reduction in their incidence. Diagnostics and treatment flowcharts are used based on the patients' complaints. It is important to note that a considerable number of STIs are asymptomatic or have nonspecific symptoms. In the case of *T. vaginalis*, for example, more than half of infected women may not exhibit any symptoms [16]; they would, therefore, not be included in the flowcharts. Moreover, there is no information about those who are diagnosed with *T. vaginalis*, since it is not a reported disease.

Prevalence studies may be useful to health professionals in their daily routines as well as for health management decisions regarding primary care. The aim of this study was to estimate the prevalence of *T. vaginalis* and its associated factors in women of childbearing age attended to by a team of family health specialists in the Federal District of Brazil.

Methodology

A cross-sectional study with consecutive sampling of women was conducted between November 2014 and March 2015. The study was conducted in the primary health care clinic of one of the 31 administrative regions of the Federal District of Brazil.

Calculation of the sample size was made taking into account a finite population of 700 individuals, a number slightly higher than the number of women of reproductive age registered in the unit. Estimating a prevalence of 10% and assuming a 5% confidence interval, the calculation indicated a sample of 116 women for the study. Predicting a dropout rate of 20%, the final sample comprised 139 women. All women between 18 and 49 years of age who visited the clinic were invited to participate. The invitation was made regardless of the reason that had led to the visit. Pregnant women and virgins were excluded. A total of 201 of 219 eligible women agreed to participate in the study. Of these, 193 were examined.

Ethical considerations

The research ethics committee of the Federal District Health Department approved the study (CAAE 28186514.5.0000.5553).

Proceedings

The women of the sample responded to a structured questionnaire administered by a trained female interviewer. They were later referred for the collection of biological samples. During the collection, vaginal pH was tested using scaled strips with a range of 3.6 to 6.1, at intervals of 0.3 units (pH-Fix, Macherey-Nagel, Düren, Germany). Simultaneously, a small amount of vaginal secretion was harvested from the fornix and placed on a glass slide with a drop of 10% potassium hydroxide to perform the whiff test. The test was considered positive when a characteristic fishy odor was noted [39].

Continuing the testing process, a sterile swab was applied against the posterior side wall of the vagina and seeded in TYM (trypticase-yeast extract-maltose) culture medium, as proposed by Diamond [40], comprising tryptone (Difco, Sparks, USA), yeast extract (Biobrás, Montes Claros, Brazil), maltose (Merck, Darmstadt, Germany), L-cysteine, chloride (Synth, Diadema, Brazil), ascorbic acid (Vetec, Rio de Janeiro, Brazil), K₂HPO₄, KH₂PO₄, distilled water. Plates were read after 24, 48, and 72 hours. After 96 hours, the culture media were washed with 5 mL of 0.9% saline solution, which was then transferred to test tubes and centrifuged at 2,500 rpm for 5 minutes. The

supernatants were discarded and the remaining pellets were examined [41]. For each sample, 100 μ L of the pellets were placed on slides and examined using an optical microscope at 40x. A second slide was made and allowed to dry at room temperature. This second slide was fixed with methanol, stained with Giemsa (Newprov, Pinhais, Brazil), and examined under a microscope at 100x. The result was considered positive when the presence of the parasite on either of the slides was verified.

Blood samples were also collected from the women and tested for syphilis, HIV, hepatitis B, and hepatitis C. Syphilis serology was performed in the regional laboratory with the use of non-treponemal (venereal disease research laboratory [VDRL]) and treponemal (TPHA) tests, according to the manufacturer's recommendations (WAMA Diagnóstica, São Carlos, Brazil). Serology for HIV as well as for hepatitis B and C were carried out at the Central Laboratory (LACEN) of the Federal District using the electrochemiluminescence (ECL) technique (Roche Diagnostics GmbH, Mannheim, Germany).

Data analysis

Data were submitted to EpiInfo7 (version 7.1.5.0, Centers for Disease Control and Prevention) and analyzed using SPSS version 22.0 (IBM, Armonk, USA). Associations between categorical variables were tested using the Chi-squared test and were measured by odds ratios (OR). Statistical significance was considered when $p < 0.05$. Differences of proportions were tested using the one-tailed Z test.

Results

A total of 219 women were invited to take part in the study. Of these, 201 women agreed to participate, answered the questionnaire, and provided blood for serology. Of these, 193 were examined by measurement of pH, the whiff test, and sample collection for *T. vaginalis* culture. The results below apply to the 193 participants who were completely examined.

The mean age of participants was 34 years (95% CI = 30.7–37.3), most of whom were of mixed ethnicity (57%). More than half of the women (53%) had eight or fewer years of schooling. Information regarding age of first sexual intercourse, smoking status, and use of hormonal contraceptives are detailed in Table 1. Thirty-

Table 1. Distribution of participating women based on sociodemographic variables, 2015.

	Women (n)	%	<i>Tv</i> prevalence % (95% CI)
Age			
18–29	75	37	14 (9–19)
30–39	65	32	18 (13–23)
40–49	61	30	15 (10–20)
Race/skin color			
Mulatto	117	58	18 (13–23)
White	49	24	11 (6–15)
Black	26	13	15 (10–20)
Others	8	4	13 (8–17)
Schooling			
4 years	38	19	19 (13–24)
5–8 years	70	35	18 (13–24)
9–11 years	91	45	12 (8–17)
Graduation	2	1	0
First sexual intercourse			
< 15 years	35	17	18 (13–24)
15–19 years	128	64	17 (12–22)
> 19 years	38	19	8 (4–12)
Smoker			
Yes	28	14	19 (13–24)
No	173	86	15 (10–20)
Hormonal contraception			
None	138	69	15 (10–20)
Oral	39	19	13 (8–18)
Injected	22	11	24 (18–30)
Other	2	1	0

Tv: *Trichomonas vaginalis*; Total N is 201, except as otherwise indicated..

three women (17%) reported having had some sexually transmitted disease (STD) at some time in their lives (Table 2).

Gynaecological complaints, predominantly lower abdomen pain (52%; 101/193), were common among participants. These were followed by reports of vaginal discharge (48%; 92/193) Table 3. Thirty samples (30/193) tested positive for *T. vaginalis* (16%; 95% CI = 11–21). Among these women, the most frequent complaint was vaginal discharge (57%). Among the women with positive cultures, 27 (90%) had at least one complaint, and 3 (10%) of them had no complaints (Table 3). Regarding age, skin color, and age of first sexual intercourse, women with positive cultures did not differ from those with negative results. In relation to the complaints, only dyspareunia was significantly higher among those with positive results.

Among the 193 women examined, 12 had positive results for hepatitis B serology (anti-HBc), but none of them were in the acute phase. All of them had negative HBsAg serology. Of these 12, two had positive results for the *T. vaginalis* culture, and. Three had positive results on the VDRL test, all confirmed by TPHA. These three participants were negative for *T. vaginalis*. Three others had positive results for HIV serology, and all were positive for *T. vaginalis*. None of the women had positive anti-HCV tests. The serology results are shown in Table 2.

The results demonstrated an association between infection with *T. vaginalis* and the total number of lifetime sexual partners. For those who had had 10 or fewer partners, this was a protective factor (OR = 0.14). Consistent condom use was also an important protective factor (OR = 0.064). There was no

Table 2. Distribution of women based on history of sexually transmitted diseases and results of serology, 2015.

	Women (n)	%	<i>Tv</i> prevalence % (95% CI)
STD report			
Yes	34	17	24 (18–30)
No	167	83	14 (9–19)
Mentioned STD (n = 34)			
Condyloma/HPV	12	35	33 (16–50)
Do not know/do not remember	10	29	29 (12–45)
Genital herpes	4	12	25 (10–40)
Gonorrhea	3	9	0
Syphilis	3	9	0
Hepatitis B	1	3	0
HIV	1	3	100
Positive tests			
VDRL (n = 200)	3	2	0
HIV (n = 195)	3	2	33
Anti-HBc (n = 194)	12	6	17

Tv: *Trichomonas vaginalis*; STD: sexually transmitted disease; VDRL: venereal disease research laboratory; Total N is 201, except as otherwise indicated.

Table 3. Distribution of women based on complaints, value of vaginal pH, and whiff test, 2015.

	Women (n)	%	<i>Tv</i> prevalence % (95% CI)
Symptoms			
Vaginal discharge	92	46	18 (13–24)
Pruritus	56	28	18 (12–23)
Burning	54	27	20 (15–26)
Bad smell	66	33	18 (13–24)
Dispareunia	68	34	22 (16–28)
Lower abdominal pain	101	50	15 (10–20)
pH value (n = 193)			
> 4.5	83	43	18 (13–24)
< 4.5	110	57	14 (9–18)
Whiff test (n = 193)			
Positive	59	31	20 (15–26)
Negative	134	69	13 (9–18)

Tv: *Trichomonas vaginalis*; Total N is 201, except as otherwise indicated.

association between positive results and the following variables: age, use of hormonal contraceptives, smoking, or gynaecological complaints. Association measures, confidence intervals, and pH values are detailed in Table 4.

Discussion

Although the Federal District is the capital of Brazil, the social and economic inequalities are quite similar to the rest of the country. The operational area of the present research was, until recently, a region considered a slum. Only in the last seven years has the region been developed with urban facilities (sewage system and paved streets). The results should be considered in light of this context.

The prevalence of *T. vaginalis* infection in this sample was found to be 16% (95% CI = 10–21). This finding was higher than that expected by the authors. A study conducted in the Federal District, (Simões-Barbosa *et al.*, 2002) found a prevalence of 7.3% in a sample of 142,158 women. Infection with *T. vaginalis* was present in 10% of the inflammatory lesions [22]. In that research, the methodology used was the Pap smear, which has sensitivity for *T. vaginalis* detection lower than that of the culture method. The disagreement

between the prevalence rates found in these two studies may be partly explained by the different methodologies. The different prevalence rates between the two populations could have an alternative explanation. The high prevalence detected in our study applies to an outpatient population. It may not reflect the prevalence in the general population. Outpatient populations may consist mostly of people with complaints or a higher perceived risk of STI. This could explain the high prevalence.

An association between education and STIs has been found by various authors [16], but educational level was not associated with positive results for *T. vaginalis* in our sample. However, more than half of the women studied had eight or fewer years of formal education, and the homogeneity of this variable could conceal possible association.

Infection with *T. vaginalis* has also been associated with variables such as age [21], smoking [18], and use of hormonal contraceptives [42]. In our study, however, there was no association between positive results for *T. vaginalis* and any of these variables.

The proportion of women with vaginal discharge was higher among those who had positive cultures (57% versus 46%); however, this difference was not

Table 4. Distribution of *T. vaginalis* infected women based on risk factors, 2015.

	OR (95% CI)	p
Hormonal contraception	1.07 (0.45–2.43)	0.87
Menstrual cycle first phase	0.83 (0.38–1.84)	0.65
Age ≥ 30 years	1.26 (0.56–2.97)	0.59
Smoking	1.28 (0.4–3.7)	0.63
Symptoms		
Vaginal discharge	1.53 (0.69–3.36)	0.29
Itching	1.27 (0.53–2.9)	0.57
Burning	1.61 (0.69–3.66)	0.26
Vaginal odor	1.34 (0.59–2.99)	0.47
Dyspareunia	2.07 (0.93–4.6)	0.07
Lower abdominal pain	0.89 (0.41–1.98)	0.78
Historical		
First sexual intercourse < 15 years of age	1.26 (0.43–3.29)	0.64
Ten or fewer partners	0.14 (0.05–0.43)	< 0.000
STD report	1.99 (0.76–4.94)	0.15
Sexual practices		
Active oral sex	0.94 (0.42–2.07)	0.88
Receptive oral sex	1.01 (0.46–2.23)	0.97
Active anilingus	0.74 (0.03–5.00)	0.87
Receptive anilingus	0.36 (0.02–2.13)	0.34
Anal	1.2 (0.41–3.14)	0.69
Vaginal douching	0.94 (0.41–2.11)	0.89
Condom use	0.064 (0.02–0.16)	< 0.000
Clinical tests		
pH > 4.5	1.39 (0.63–3.08)	0.4
Positive whiff test	1.64 (0.72–3.68)	0.23

statistically significant. Similarly, the complaints of pruritus, burning, malodorous secretion, and dyspareunia, although not significant, were also proportionally more common among women with positive results for *T. vaginalis*. On the other hand, the difference between the proportions in terms of those who mentioned pain during sexual intercourse was significant. Generally, among the culture-positive women, genital complaints were frequent; this also occurred among the culture-negative women. In other words, although infected women were not completely asymptomatic, the symptoms reported were not specific to *T. vaginalis* and may not be attributed solely to this infection. Moreover, 10% of women with positive cultures were completely asymptomatic.

The pH values tend to be above 4.5 in women infected with *T. vaginalis*, and the whiff test is usually positive [43]. In our study, 50% of infected women had pH values above 4.5, and 40% were positive in the test of the amines. Considering pH, although the prevalence of infection was higher in women with values above 4.5 (18% versus 14%), the association was not significant. A similar observation applied to whiff test: the prevalence among the positive and negative women was, respectively, 20% and 13%, without statistical significance. A potential limitation of our study is the fact that a large proportion of the participants (43%) made regular use of vaginal douches. This may have compromised the results of the pH and the whiff tests. Another potential limitation was the sample size used for this study.

There was no association between prior STI reported and positive cultures. Interpretation of this result requires a degree of care for two reasons: 1) among women who reported having had an STI, nearly one-third did not reveal the diagnosis; some common genital changes may be misclassified as an STI; and 2) women with an STI do not always have an adequate perception of their condition; there may have been incidences of STIs among those who denied prior STI history.

A few women had positive results for syphilis, hepatitis B, and HIV. This situation limits the potential of statistical tests. An association between *T. vaginalis* and number of lifetime sexual partners was found in another study [44] and is not surprising for this STI. Similarly, the protective action of consistent condom use was expected [45] and corroborates the recommendation for condom use, especially among people who have no steady partner or have more than one sexual partner.

Conclusions

The prevalence of *T. vaginalis* infection was considered high in the sample examined. The infection was positively associated with the number of lifetime sexual partners, and consistent condom use was a protective factor. Vaginal complaints were more common among women with *T. vaginalis*, but only dyspareunia was significantly higher.

Replication of this study in other administrative regions of the Federal District may provide important information for understanding the magnitude of this disease in populations; its distribution in different genres, age groups, and social strata; and the impact on quality of life. Such information can be useful to the managers responsible for public health in the Federal District.

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