

# TRICHOMONAS VAGINALIS INFECTION AMONG WOMEN ATTENDING IN THE PUBLIC SERVICE IN RIO GRANDE DO SUL, BRAZIL: FREQUENCY, RISK FACTORS AND CLINICAL SIGNS

*INFECÇÃO POR TRICHOMONAS VAGINALIS EM MULHERES ATENDIDAS PELO SERVIÇO  
PÚBLICO NO RIO GRANDE DO SUL, BRASIL: FREQUÊNCIA, FATORES DE RISCO E SINAIS CLÍNICOS*

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## ABSTRACT

**Introduction:** Trichomoniasis is a cosmopolitan disease that can affect the female fertility, and is commonly underdiagnosed, both in private practices and in public health services, because of the low sensitivity of the wet mount exam used routinely. **Objective:** To understand the occurrence of *T. vaginalis* infection by means of *in vitro* culture in women receiving care in a public health unit, in the city of Pelotas, Rio Grande do Sul, Brazil, as well as to identify the possible risk factors associated with this infection. **Methods:** Cross-sectional study was carried out, which included 201 women undergoing interview and gynecological exam, with the collection of vaginal discharge in the Gynecology Ambulatory at the School of Medicine of Universidade Federal de Pelotas. The material collected was examined in the form of fresh smears and cultivated in Diamond's medium. Epidemiological data were obtained by means of patient interviews and clinical trials, from the medical records. The results were statistically analyzed through  $\chi^2$  and Fisher's exact tests, using version 9.0 of the Statistix program. **Results:** The occurrence of *T. vaginalis* infection was 7% (14/201). In the wet mount exam, used routinely for the diagnosis of this agent, only 42.85% of the infected women (6/14) were positive. It was noted that 21.4% of the infected women were asymptomatic, and 89.05% were not aware of the existence of the *Trichomonas vaginalis* infection. Factors independently associated with the infection were the smoking habit (odds ratio [OR] = 11.8), not having a stable sexual partner (OR = 6.36), presence of vaginal discharge with odor (OR = 5.65), and altered vaginal microbiota (OR = 5.31). **Conclusion:** *T. vaginalis* infection was present among the women studied, being underestimated because of the diagnostic technique, and because many of them were asymptomatic. The smoking habit, not having a stable sexual partner, having fetid discharge, and altered vaginal microbiota are the risk factors for infection.

**Keywords:** *Trichomonas vaginalis*; diagnosis; Trichomonas infections; risk factors.

## RESUMO

**Introdução:** A tricomoníase é cosmopolita, pode afetar a fertilidade feminina, e geralmente é subdiagnosticada, tanto em consultórios particulares, quanto em serviços públicos, devido à baixa sensibilidade do exame a fresco, usado rotineiramente. **Objetivo:** Conhecer a ocorrência de infecção por *Trichomonas vaginalis*, através de cultivo *in vitro*, em mulheres atendidas em unidade pública, na cidade de Pelotas, Rio Grande do Sul, além de identificar os possíveis fatores de risco associados a essa infecção. **Métodos:** Estudo de corte transversal que incluiu 201 mulheres, submetidas a entrevista e exame ginecológico, com coleta de conteúdo vaginal, no Ambulatório de Ginecologia da Faculdade de Medicina da Universidade Federal de Pelotas. O material coletado foi examinado a fresco e cultivado em meio de Diamond. Foram obtidos dados epidemiológicos através de entrevista, e clínicos, nos prontuários. Os resultados foram analisados estatisticamente através dos testes do  $\chi^2$  e exato de Fisher, utilizando o programa Statistix versão 9.0. **Resultados:** A ocorrência da infecção por *T. vaginalis* foi de 7% (14/201). No exame a fresco, usado como rotina para o diagnóstico desse agente, apenas 42,85% das infectadas (6/14) foram positivas. Constatou-se que 21,4% das mulheres infectadas são assintomáticas, e que 89,05% desconheciam a existência da tricomoníase. Os fatores independentemente associados com a infecção foram o hábito de fumar (OR=11,8), não ter companheiro fixo (OR=6,36), apresentar corrimento vaginal fétido (OR=5,65) e microbiota vaginal alterada (OR=5,31). **Conclusão:** A infecção por *T. vaginalis* está presente entre as mulheres estudadas, e sendo subestimada, devido à técnica de diagnóstico e por muitas serem assintomáticas. O hábito de fumar, não ter companheiro fixo, ter corrimento fétido e microbiota vaginal alterada são fatores de risco para a infecção.

**Palavras-chave:** *Trichomonas vaginalis*; diagnóstico; tricomoníase; fatores de risco.

## INTRODUCTION

*Trichomonas vaginalis* as well as trichomoniasis were described nearly 200 years ago (1836) and trichomoniasis remains the most prevalent nonviral sexually transmitted disease (STD) in the world. The protozoan causes a disease that has a relatively simple and low-cost treatment, but factors such as populational misinformation and the fact that most of the infected individuals are asymptomatic and underdiagnosed explains its wide geographic distribution. Its importance has recently been recognized, as it can cause reproductive complications and facilitate infection with human immunodeficiency virus (HIV)<sup>(1)</sup>.

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The World Health Organization estimates that there are 276.4 million new cases per year worldwide<sup>(2)</sup>. Prevalence studies indicate that data vary according to the diagnostic techniques used, the presence of other STDs, and the socioeconomic conditions of the population studied among others. In Brazil, these prevalences range from 1.9 to 37.5%<sup>(3)</sup>.

The infection by *T. vaginalis* can cause vulvovaginitis, characterized by the presence of yellowish-white discharge, often fetid with the presence of bubbles, and erythematous vaginal and cervical walls. However, about half of the women infected are asymptomatic, and the infection can persist for an average of 3 to 5 years. These characteristics aggravate the problem by allowing the transmission to subsequent partners, and the fact that about a third of them become symptomatic in up to 6 months makes it difficult to establish the source of infection<sup>(4)</sup>.

The diagnostic routine, performed both in private gynecological clinics and in public health services, is usually based on clinical examination, which is difficult as the symptoms are common to other STDs. Laboratory investigation is needed, and is made through direct examination of the vaginal exudate collected during the pelvic examination under light microscopy, during which the motility of living protozoa is perceived<sup>(4)</sup>. However, the sensitivity of this technique is low, diagnosing the infection in only 30 to 60% of the women infected<sup>(5)</sup>. Thus, in many undiagnosed and untreated women, the infection may be aggravated, and they become more susceptible to infection by HIV<sup>(1)</sup>, spreading the protozoan to sexual partners, as well as being more likely to have reproductive complications.

In clinical centers, where more improved diagnostic techniques such as *in vitro* culture are offered, which are 70–80% more sensitive than the wet mount technique, the visualization of the parasite is facilitated because of its multiplication<sup>(6)</sup>. In this technique, the samples are incubated under optimal conditions for 5 to 7 days, and majority of clinics do not have easy access to this diagnosis<sup>(7)</sup>. On the other hand, a commercial kit, known as InPouch TV, representing the traditional culture method, is so efficient and sensitive that it can be used in laboratories<sup>(2)</sup>. Molecular techniques, such as the nucleic acid amplification test (NAAT), have 100% sensitivity, but are still not viable as a routine, because it requires special equipment and reagents, technical expertise, and has high cost<sup>(2)</sup>.

Studies conducted in different countries found different factors related to *T. vaginalis* infection in women. Among them are older age, black ethnicity, higher number of sexual partners in the previous year, prostitution, having same-sex partners, using illicit drugs, not using condoms, having other STDs, and having low education levels<sup>(8-12)</sup>.

## OBJECTIVE

This study aimed at assessing the occurrence of infection with *T. vaginalis* through *in vitro* culture in women seeking care in a public unit in the city of Pelotas, Rio Grande do Sul, as well as at identifying the possible risk factors associated with this infection and the main related medical conditions.

## METHODS

The prospective study was conducted at the Clinic of Gynecology and Obstetrics in the Infant and Maternal Department of the School of

Medicine (FAMED) of the Universidade Federal de Pelotas (UFPe), which is a reference center for the diagnosis of STDs in the region. This center provides service to an average of 40 to 50 cases daily, through the Unified Health System. These correspond to the gynecologic procedures (including wet mount test with vaginal content for the diagnosis of *trichomonas* infections), prevention of gynecologic cancers, surgical pathologies, mastology, and the prenatal care for low- and high-risk pregnancies. A sample of each patient was taken to the Parasitology Laboratory of the Biology Institute of the same university, for the diagnosis of *T. vaginalis* through *in vitro* culture. The study was approved by the Research Ethics Committee of the FAMED of the UFPe (Protocol No. 873.180).

A total of 201 patients were analyzed during the period from January to October 2015. Patients awaiting consultation were invited to participate. After clarification of the project and acceptance by the patients, an informed consent was signed, based on Resolution No. 466/12 of the National Health Council of the Brazilian Ministry of Health, for the authorization of the collection of vaginal content during gynecological examination, as well as for the access to the data contained in the medical records. Afterward, they were interviewed by a researcher in a private environment, without witnesses, in secrecy and confidentiality. They answered a questionnaire covering demographic data, sexual habits, symptoms, knowledge of trichomoniasis, and use of condoms, among other details.

In addition to gynecological clinical examination, they were subjected to the collection of material for cytological diagnosis for the prevention of cervical cancer, routinely performed by the accredited laboratory at the clinic. During some consultations, rapid detection tests were also performed for the diagnosis of STDs, such as HIV, hepatitis B and C, and syphilis. In this study, we used cotton swabs and Ayre spatula, with the aid of a speculum, to collect vaginal and cervical content. The diagnosis of *T. vaginalis* was done through direct examination of wet mounts, used routinely in the clinic, and also by *in vitro* culture of the parasite. To perform the wet mount test, a training was given to perform the readings, which were read alternately by two responsible researchers. The examination was performed immediately after the collection of the material that was smeared on to a microscope slide with Brilliant Cresyl Blue solution (to facilitate observation), and covered with a coverslip, then observed through a binocular optical microscope under 40x objective lens. For culture, the swab with the collected material was immediately introduced into an Eppendorf tube containing the Diamond culture medium and transported to the Parasitology Laboratory in an isothermal box. Samples were cultured in Diamond's medium (trypticase–yeast extract maltose – TYM), pH 6.0, supplemented with inactivated 10% adult bovine serum, plus antibiotic and antifungal agents. In the laboratory, the material was homogenized and transferred to 15 mL Falcon tubes containing 9 mL of the same medium, incubated vertically at 37°C; daily observations were made in the microscope, and only after 7 days, the material was considered negative. In positive samples, the motility of protozoa was clearly observed.

Patients for whom the wet mount test was positive, and for those whose result was negative but who showed clinical signs characteristic of trichomoniasis during the consultation, received free treatment with oral or vaginal metronidazole to treat the infection. The patients

whose wet mount was negative, but the culture was positive, were called to receive treatment. Information relating to the positive result of the culture was transferred to the medical records of these patients.

Data were organized using spreadsheets in Excel (Microsoft). First, the independent variables (risk factors for *T. vaginalis*) were subjected to univariate analysis to assess the association of each of the possible risk factors and the dependent variable (positive culture for *T. vaginalis*) through the  $\chi^2$  and accurate Fisher's tests. The cut-off point adopted at this stage of the analysis was  $p < 0.25$ , and the associated factors were then evaluated in the logistic regression or multivariate analysis. The model was built using elimination in descending order according to the p-value. The variables with  $p \leq 0.05$  were maintained until the end of the multivariate analysis. For the study, the Statistix 9.0 software was used. The odds ratio (OR) was calculated with a 95% confidence interval (95%CI).

## RESULTS

Among the 201 women surveyed, 14 (7%) were infected with *T. vaginalis*. This result was obtained by *in vitro* culture. In the wet mount test (routinely used), the parasite was detected in only 42.85% of the infected patients (6/14).

The data on the sociodemographic profile and the relationship with trichomoniasis of the patients in the study are shown in **Table 1**. This is a sample population with low levels of income and education, and in most cases, they were 21 to 50 years of age, white, and had a stable partner.

Factors analyzed and their respective frequencies, which were the significant predictors of *T. vaginalis* infection in the univariate analysis ( $p < 0.25$ ), are shown in **Table 2**. It was found that 89.05% of the women reported no knowledge of the disease.

After application of the multivariate logistic regression model, it was found that the factors that have a statistically significant relationship ( $p = 0.05$ ) with the infection were smoking, not having a steady partner, having vaginal discharge with foul odor, and altered vaginal microbiota because of the decrease or absence of Döderlein bacilli. The risk factors associated with the infection by *T. vaginalis* and their respective OR are shown in **Table 3**.

As for the symptoms presented, it was found that 78.6% (11/14) of the infected women had clinical signs of the disease. The most common signs in these patients were white or yellow vaginal discharge (90.9%), vaginal itching (54.5%), vaginal discharge with foul odor (45.5%), and painful urination (9.1%). **Figure 1** shows the difference in frequency of these symptoms among women infected and uninfected by *T. vaginalis*.

Other infections have been diagnosed during the study, such as those caused by fungi (*Candida*), viruses (HIV, human papillomavirus [HPV], hepatitis C, and herpes infections), or bacteria (syphilis and *Gardnerella vaginalis* according to Amsel's criteria), through the wet mount test or through quick detection tests. However, there was no statistically significant difference between the frequency of these infections in women infected or uninfected by *T. vaginalis*. In the population studied, 48.3% (97/201) were infected by at least one of the above said agents.

## DISCUSSION

Most studies held in Brazil on the prevalence of infection by *T. vaginalis* were carried out through the diagnosis by wet mount test or cytopathology, the routine techniques for the diagnosis of parasitosis. Prevalence rates in these studies vary from 0.6% in women of Alta Sorocabana, São Paulo<sup>(13)</sup> to 10.5% in women of

**Table 1** – Sociodemographic profile of 201 patients surveyed at the Clinic of Gynecology of the School of Medicine of Universidade Federal de Pelotas, Rio Grande do Sul, between January and October 2015, and its relationship with infection by *Trichomonas vaginalis*.

Characteristics	Frequency		Tv positive (n=14)	Tv negative (n=187)
	n	%	n (%)	n (%)
Age				
>40 years	90	44.77	5 (35.7)	85 (45.5)
≤40 years	111	55.22	9 (64.3)	102 (54.5)
Ethnicity				
White	143	71.14	7 (50.0)	136 (72.7)
Black/brown	58	28.85	7 (50.0)	51 (27.3)
Marital status				
Married	141	70.14	7 (50.0)	134 (71.7)
Single, separated, divorced, widowed	60	29.85	7 (50.0)	53 (28.34)
Education				
Complete high school or more	72	35.82	4 (28.6)	68 (36.4)
Incomplete high school or less	129	64.17	10 (71.4)	119 (63.6)
Income				
>1 minimum wage	31	15.42	5 (35.7)	26 (13.9)
≤1 minimum wage	170	84.57	9 (64.3)	161 (86.1)
No. of people living in the same residence				
≤5 people	185	92.03	13 (92.9)	172 (92.0)
>5 people	16	7.96	1 (7.1)	15 (8.0)

Tv: *Trichomonas vaginalis*.

**Table 2** – Frequency of significant risk factors predictive of *T. vaginalis* infection in the univariate analysis ( $p < 0.25$ ) in women treated at the Clinic of Gynecology of the School of Medicine of Universidade Federal de Pelotas, in southern Rio Grande do Sul ( $n=201$ ).

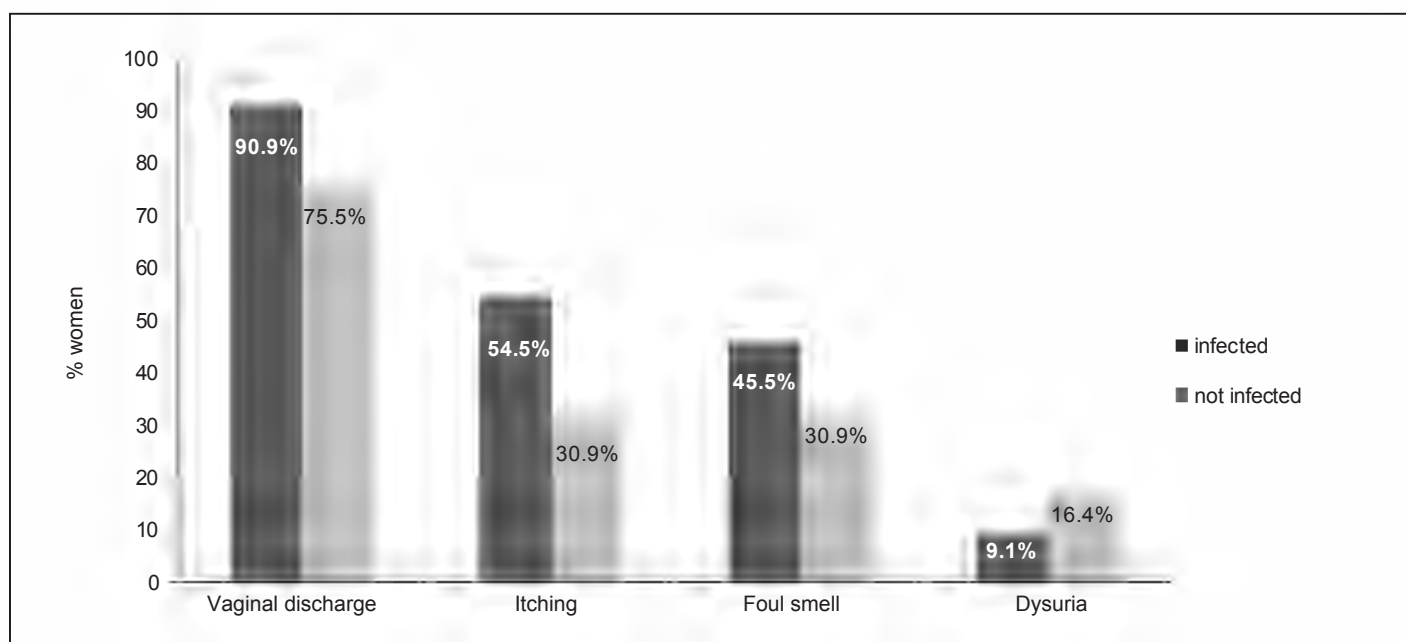
Variables	Frequency		Diagnosis				p-value
	n	%	Positive		Negative		
			n	%	n	%	
Being a smoker	59	29.35	9	15.25	50	84.74	0.0029
Itching	40	19.90	6	15.00	34	56.66	0.0257
Unawareness of trichomoniasis	179	89.05	11	6.14	168	93.85	0.1927
Black or mixed ethnicity	58	28.85	7	12.06	51	87.93	0.0703
Vaginal discharge	92	45.77	9	9.78	83	90.21	0.1494
No fixed sexual partner	60	29.85	7	11.66	53	88.33	0.0876
Altered vaginal microbiota	120	59.70	12	10.00	108	90.00	0.0397
Income up to 1 minimum wage	170	84.57	9	5.29	161	94.70	0.0293
Being in menopause	45	22.38	1	2.22	44	97.77	0.1560
Having fetid odor	39	19.40	5	12.82	34	87.17	0.1096
Having other STDs	86	42.78	3	3.48	83	96.51	0.0940

STDs: sexually transmitted diseases.

**Table 3** – Factors significantly associated with infection by *T. vaginalis* in patients treated at the Clinic of Gynecology of the School of Medicine of Universidade Federal de Pelotas, Rio Grande do Sul, between January and October 2015 (multivariate analysis,  $p \leq 0.05$ ).

Characteristics	Positive		Negative		OR	95%CI	p-value
	n	%	n	%			
Being a smoker	9	64.3	50	26.7	11.84	2.5–55.8	0.0018
No fixed sexual partner	7	50.0	53	28.3	6.36	1.4–28.9	0.0166
Vaginal discharge with foul smell	5	35.7	34	18.2	5.65	1.2–26.2	0.0270
Altered vaginal microbiota	12	85.7	108	57.8	5.31	1.0–29.2	0.0545

OR: odds ratio; 95%CI: 95% confidence interval.

**Figure 1** – Frequency of major symptoms in women treated at the Clinic of Gynecology of the School of Medicine of Universidade Federal de Pelotas, Rio Grande do Sul, infected and not infected with *T. vaginalis*, from January to October 2015.



Vitória de Santo Antônio, Pernambuco<sup>(14)</sup>. In this study, the infection by protozoan was detected in 3% (6/201) of women through a wet mount test. This is a low-sensitivity diagnostic technique, as it needs a higher concentration of the parasite in the vaginal content so that they can be observed. However, when *in vitro* culture was performed for this diagnosis, it was found that 7% (14/201) of the women were infected. This result corroborates with the results of the studies by other authors in Brazil, who, using this technique, found prevalence rates ranging from 2.5<sup>(15)</sup> to 13.5%<sup>(16)</sup>, with an average of 5.8%. But when female prisoners<sup>(8)</sup> or women living in extreme poverty<sup>(17)</sup> were examined, these rates reached between 30 and 20%, respectively, showing the importance of hygiene, cultural, and behavioral habits to fight this parasitosis. Infection rates can also be influenced by factors such as the stage of menstrual cycle at which the collection is made<sup>(3)</sup>.

The *in vitro* culture is considered the gold standard for the diagnosis of trichomoniasis, for its high sensitivity, and for the difficulty and cost of molecular techniques<sup>(6)</sup>. However, it is still not used as routine diagnosis, as trichomoniasis is a neglected disease that receives little attention from health agencies, despite being widely disseminated and representing a public health problem.

The sociodemographic profile of the women studied, most with low education and income levels, is due to the fact that the study was conducted in a clinic from the public health service. The most frequent age group, 21 to 50 years, is exactly that of sexually active women who often seek gynecological care. The large percentage of women who are unaware of the disease (89.05%) makes them more vulnerable to acquiring the infection, as well as other STDs.

The risk factors, most often associated with the infection as cited in the literature, in the order of frequency are having more than one sexual partner in the last 3 months, not using condoms, black ethnicity, older than 40 years, low education, illegal drug use, smoking, alcoholic, and partner with low education level<sup>(8,12,18,19)</sup>. In this study, it was found, by bivariate analysis, that the factors that were significant predictors of infection by *T. vaginalis* were smoking, being black or brown, not having a steady partner, income below the minimum wage, being in the menopause, unaware of the disease, presenting vaginal discharge, itching or foul odor, having altered vaginal microbiota, and other STDs, such as herpes, HPV, syphilis, hepatitis B and C, and HIV. The clinical and pathological factors, already reported by other authors, were the existence of other STDs, altered vaginal microbiota, symptoms such as itching, vaginal discharge, foul smell, dysuria, dyspareunia, and pelvic pain<sup>(11,14)</sup>. It is evident in this and other studies that the economic factor is relevant, as it interferes with education, ignorance of STDs, and consequent oversights in prevention.

Among the risk factors, the one that is statistically associated with infection (logistic regression) is smoking, which increases the risk of infection up to 11.48 times, as observed by other authors<sup>(20-23)</sup>. It was also found that women who have no steady partner had 6.36 times greater risk of acquiring the infection than those who have, a factor of disagreement between authors, because although some agree with this information<sup>(24)</sup>, as the first group of women generally have a greater number of partners making them more exposed to STDs, other authors have found higher prevalence rates among those with a steady partner<sup>(18)</sup> and who claim to trust their partner and who

do not use condoms. The presence of vaginal discharge with foul smell is a strong clinical evidence of this infection, and women with this symptom were 5.65 times more likely to be infected, confirming with the results of other findings<sup>(14,20)</sup>. Another associated factor was the presence of altered vaginal microbiota (OR=5.31), as the protozoan causes vaginitis. It has also been reported as a risk factor by other authors<sup>(25)</sup>. The inflammatory reaction caused by *T. vaginalis* generally favors the installation of anaerobic bacterial vaginitis, responsible for the release of amines that produce foul odor. Also because of the inflammatory reaction, there is the accumulation of leukocytes in the vaginal content, observed even in the wet mount test.

Many patients infected with *T. vaginalis* are asymptomatic, which further complicates the control of the disease, as they are a source of infection, being exposed to the complications of the infection, and not being induced to seek medical help. In the group studied, 21% of the infected women were asymptomatic. This percentage may vary from 10 to 50%, even if *in vitro* culture is used as a diagnostic technique<sup>(20)</sup>. Several factors can interfere in these indices, such as the time between the collection and the culture of material, its precision, the absence of secondary contamination that may prevent the growth of protozoa, and the population studied.

The most common symptoms found in women infected with *T. vaginalis* (yellowish-white discharge, foul odor, and itching) coincide with those described by other authors<sup>(6,14,20)</sup>. Although they occur in other gynecological infections, they are strong indications for the clinical diagnosis of trichomoniasis, which was confirmed in this study by the higher frequency of these symptoms among patients infected by the protozoan.

The fact of being infected with trichomoniasis or not did not significantly alter the frequency of other infections diagnosed during the study (candidiasis, HIV infections, HPV infections, hepatitis C, syphilis, and *Gardnerella vaginalis*). However, in other studies, it was found that women with genital itching and those diagnosed with syphilis, herpes, *Chlamydia trachomatis*, or *Mycoplasma genitalium* were at a risk for infection with *T. vaginalis*<sup>(22)</sup>. It has also been found that the HIV infection facilitates the infection of *T. vaginalis* and vice versa<sup>(5)</sup>. In fact, the presence of an STD indicates exposure to the diagnosed agent and also to other agents that can be transmitted in the same way.

## CONCLUSION

Infection by *T. vaginalis* is prevalent among the women studied, many of whom were asymptomatic. This is an underdiagnosed infection because, besides the fact that there is a great ignorance among the patients about the infection, the routine diagnostic technique (wet mount test) is not very sensitive, and the culture is still a laborious and not a very accessible technique. Smoking, not having a steady partner, presenting vaginal discharge with foul odor, and abnormal vaginal microbiota are associated with the increased risk for this infection. Ignorance of the disease, high number of infected women, asymptomatic patients, and difficulties in the deployment of more sensitive diagnostic techniques reveal an alarming picture on trichomoniasis, which requires public educational measures and changes in the routine diagnosis.

## Conflict of interests

The authors report no conflict of interests.

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