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ORIGINAL ARTICLE

Prevalence and Risk Factors for *Trichomonas Vaginalis* Infection Among Women: a Population-Based Controlled Study in Saudi Arabia

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SUMMARY

Background: **Information on *Trichomonas vaginalis* (*T. vaginalis*) infection in Saudi Arabia is scarce. The aim of study was to assess the burden and risk factors of *T. vaginalis* infection for a cohort of women living in Saudi Arabia.**

Methods: **Women aged ≥ 18 years who were seeking medical care at the King Faisal Medical Complex Gynecology Clinic in Taif city, Western Saudi Arabia, were enrolled in a non-randomized case-control study between June 2018 and May 2019. Participants were interviewed using a standard questionnaire for a number of socio-demographic and clinical characteristics. Vaginal swabs obtained from each participant were screened for *T. vaginalis* infection with direct wet mount smear microscopy, the OSOM Trichomonas rapid test 'OSOM Trich' (Genzyme Diagnostics, Cambridge, MA, USA) and a published nested PCR.**

Results: **Over the study period, 155 women were recruited: 79 with symptoms of vaginitis (i.e. cases) and 76 with no symptoms (i.e. controls). The *T. vaginalis* infection was detected in ~20% (16/79) of cases and ~9% (7/76) of the controls by the nested PCR. Using the PCR test results as a gold standard, the wet mount microscopy's sensitivity, specificity, negative predictive value, and positive predictive value were 69.5%, 100%, 94.9%, and 100%, respectively, whereas the OSOM Trich's were 86.9%, 100%, 97.7%, and 100%, respectively. The main high-risk factors included age between 30 and 39 years (~35%), marriage for 10 - 30 years (~62%), non-education (~41%), urban residence (~29%), and employment (~36%). Highly significant differences were observed concerning infection distribution among cases for the presence of lower abdominal pain (~64%) and abnormal vaginal discharge (38%) as presenting symptoms ($\chi^2 = 20.42$; $p < 0.001$ and $\chi^2 = 5.63$; $p = 0.017$, respectively).**

Conclusions: **The burden of infection with *T. vaginalis* is unexpectedly high in the population studied. Regular screening for *T. vaginalis* infection, particularly in high-risk women, is required.**

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INTRODUCTION

Trichomonas vaginalis (*T. vaginalis*) is a strictly human parasite of worldwide distribution. The parasite presents itself as pear-shaped, motile, nucleated, and flagellated cell colonizing the male and female urogenital systems. The World Health Organization reported *T. vaginalis* as the first in a series of curable sexually transmitted infections, with annual incidence of ~250 million reported cases [1].

Infection in women is asymptomatic in almost half of cases; however, infected women can experience symptoms like malodorous vaginal discharge, vulvar irritation, and dysuria. Chronic non-treated infection in women can predispose to infertility, pelvic inflammatory disease, and cancer of the cervix [2]. Male infection is mostly asymptomatic, but, infected men may experience urethritis, urethral discharge, and dysuria. Chronic non-treated infection in men can cause prostatitis, infertility, and an increased incidence of prostate cancers [3]. The parasite has recently gained increasing attention due to the role it could play in increasing the transmission risk of the human immunodeficiency virus (HIV) [4].

Trichomoniasis is difficult to diagnose clinically, not only because the symptoms are similar to other vaginal infections, but also because of the difficulties and limitations associated with the diagnostic methods routinely used, namely wet mount microscopy and vaginal discharge culture [5]. To circumvent the time- and labor-intensive use of cultures, novel, more reliable, inexpensive, and convenient rapid diagnostic tests and nucleic acids amplification assays have been recently developed to detect *T. vaginalis* infection in distinct genitourinary clinical samples [6].

Due to cultural and religious considerations, data on sexually transmitted infections, including trichomoniasis, are not well understood in Saudi Arabia. Culture and community are not fully committed to the idea of being infected with sexually transmitted infections. In addition, *T. vaginalis* is not a reportable infection for public health officials and is generally not part of routine screening in the country. There is therefore little information on the prevalence of *T. vaginalis* in Saudi Arabia. In addition, studies on infection burden were conducted 1-3 decades ago on the basis of relatively insensitive methods [7,8].

The aim of our study was to assess the occurrence of *T. vaginalis* infection through conventional and molecular diagnosis among symptomatic and asymptomatic women seeking medical care at a Gynecology Clinic in the city of Taif, Western Saudi Arabia, as well as to identify possible risk factors associated with this infection.

MATERIALS AND METHODS

Study site

Women interviews, examination and vaginal swabs sample collection were carried out at King Faisal Medical Complex (KFMC) Gynecology Clinic in Taif, Saudi Arabia from June 2018 to May 2019. Parasitological examination of samples was performed in the Microbiology Department of KFMC. Molecular diagnosis was done at the Medical Laboratory Department at the College of Applied Medical Sciences, Taif University.

Study design

This is a comparative case-control study, where women ≥ 18 years with vaginitis-related symptoms were enrolled in the study as cases. This group attended the Gynecology Clinic for treatment. Almost the same number of asymptomatic women were chosen, matched by age and gender, to represent the control group. They attended the same clinic for participation in a screening program.

Criteria for exclusion

Women who were pregnant, menstruating, receiving anti-parasitic treatment, had intercourse within three days of the interview, used topical or oral anti-trichomonas vaginal therapy within the previous 15 days were excluded from the study.

Data collection

An interview questionnaire was designed to collect the data from all participants. The data included a number of sociodemographic features (age, residence, nationality, education level), sexual history (marital status, and duration of marriage) and clinical complaints (vaginal discharge, itching, irritation, burning, polyuria, dysuria, lower abdominal pain, and/or dyspareunia)**.**

Ethical consideration

This research was accepted by the Institutional Review Board of the College of Applied Medical Science and approved by the IRB (Ref.: KFMC-02-T-067) for the KFMC parent clinical trial. A signed consent was obtained from each participant prior to enrollment. Women with reported trichomoniasis were informed and treated by the clinician.

Vaginal swabs collection

Vaginal swab samples, three per patient, were obtained by the gynecologist. The first swab was held in a tube containing 3 mL of sterile phosphate buffered saline (PBS, pH 7.2) for direct wet mount microscopy. The second swab was kept in a test tube containing 1 ml of sample extraction buffer and stored at 4°C for up to 24 hours for antigen detection testing. The last swab was kept frozen (at -20°C) for PCR-based molecular diagnosis.

Detection of infection

Three distinct methods (wet mount microscopy, rapid antigen detection, and nested PCR) were adopted in this study for detection of *T. vaginalis* in vaginal swab samples.

Wet mount microscopy

One vaginal swab was mixed with 0.5 mL of normal saline immediately, and a drop of the mixture was placed on a clean glass slide with a cover slip and examined microscopically for motile *T. vaginalis* under × 10 and × 40 objectives, as mentioned elsewhere [9].

Rapid test kit

The OSOM *Trichomonas* Rapid Test Kit (Genzyme Diagnostics, Cambridge, MA, USA) was performed for the qualitative detection of *T. vaginalis* antigens in vaginal swabs. OSOM Trich is an immunochromatographic capillary-flow enzyme immunoassay dipstick test that was performed according to the manufacturer's instructions. Vaginal swabs were individually-tested, and analysis required 10 to 15 minutes. A positive result has both a red internal control and a blue positive test line, while the negative result has only a red internal control line. Invalid tests had an absent internal control line.

Trichomonas vaginalis nested-PCR

The frozen swab samples were removed from the freezer and left to thaw at room temperature. After thawing, samples were first centrifuged for 5 minutes at 5,000 rpm then subjected to genomic DNA (gDNA) extraction using the QIAamp DNA mini kit (QIAGEN, Hilden, Germany) in accordance with the manufacturer's instructions. Quantity and purity of the extracted DNA was measured with Nanodrop (ThermoScientific NanoDrop 1000 spectrophotometer, USA) while, the quality of extracted DNA was tested by 1.5% agarose gel electrophoresis. A species-specific nested PCR was performed using two sets of primers (OUT-F and OUT-R / IN-F and IN-R) targeting sequence of 1,100 bp of the *T. vaginalis* actin gene [10]. The PCR reactions' set up was adopted in a final volume of 50 µL with reagent concentrations closely similar to that mentioned in the original protocol. The PCR reactions and cycling conditions were performed as described in detail elsewhere [11]. The amplification reactions were conducted using an Eppendorf gradient thermocycler. Finally, the PCR amplicons were visualized and analyzed on ethidium bromide-stained 1.5% agarose gel electrophoresis.

Statistical data analysis

Data were collected, tabulated, and analyzed in SPSS, version 21 (IBM SPSS Statistics for Windows; IBM Corp., Armonk, NY, USA). The association between categorical variables were performed using the chi square (χ^2) test. Results were considered statistically significant if $p < 0.05$. The diagnostic performance of both wet mount microscopy and the OSOM Trich test was calculated based on the nested PCR test findings, as previously described [12]. Calculation of the diagnostic performance parameters (sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV)) of distinct tests, was performed using an online free web site:
https://www.medcalc.org/calc/diagnostic_test.php.

RESULTS

Figure 1 shows the women's recruitment flowchart. Over the study period, a total of 643 women were invited to take part in the current research. Of these, 256 women agreed to participate and were subjected to eligibility's criteria. As a result 101 women were excluded from participation and 155 women were recruited in the current study.

The sociodemographic and clinical features of the studied sample

Out of the 155 women recruited in the study, 79 were symptomatic (cases) with a median age of 42 (23 - 49) and 76 were asymptomatic with a median age of 46 (18 - 52) years. The majority of cases were non-Saudi residents (62%) while most of the controls (71%) were Saudi citizens. The other demographic characteristics of both cases and controls are listed in Table 1. The presence of abnormal vaginal discharge (26.5), lower abdominal pain (17.7%), vulval itching (16.4%), burning sensation (11.3%), odor (10.1%), dysuria (10.1%), and dyspareunia (7.5%) were the presenting symptoms for cases.

The burden of infection among cases and controls

Table 2 shows the burden of infection in the studied women cohort according the diagnostic method used. The direct wet mount microscopy successfully identified *T. vaginalis* trophozoites in 16 (10.3%) vaginal swab specimens: 15 cases (18.9%) and one control (1.3%). The difference between the two cohorts (cases and controls) was statistically highly significant ($\chi^2 = 13.06$, $p < 0.001$). The OSOM Trich test identified the *T. vaginalis* antigen in 20 (12.9%) vaginal swab samples: 15 cases (18.9%) and 5 controls (6.5%). The difference was statistically significant ($\chi^2 = 5.86$, $p = 0.015$). Samples with discordant results between microscopy and the OSOM Trich test were subjected to DNA extraction and PCR amplification. The nested PCR assay identified the target DNA sequence of *T. vaginalis* in these samples. Moreover, the nested PCR assay succeeded to pick up one positive case (16/79; 20.2%) and two positive controls (7/76; 9.2%) over the rapid diagnostic test. The target DNA sequence of *T. vaginalis* was successfully identified in 20.2% (16/79) of cases compared to 9.2% (7/76) of the controls, with a recorded overall prevalence rate of 14.8% (23/155). This difference was statistically insignificant ($\chi^2 = 3.73$, $p = 0.053$). Importantly, all swabs that were identified as negative for *T. vaginalis* trophozoites and/or for the

Table 1. Characteristics of 155 studied women over the period between June 2018 and May 2019.

Characteristic	Case (total = 79) n (%)	Control (total = 76) n (%)
Demographics		
Age per years		
Median (IQR)	**42 (23 - 49)**	**46 (18 - 52)**
18 - 29 years	**25 (31.6)**	**32 (42.1)**
30 - 39 years	**37 (46.8)**	**29 (38.1)**
≥ 40 years	**17 (21.5)**	**15 (19.7)**
Duration of marriage		
< 10 years	**49 (62.0)**	**52 (68.4)**
10 - 30 years	**13 (16.4)**	**9 (11.8)**
> 30 years	**17 (21.5)**	**15 (19.7)**
Education level		
High (> 12 years)	**21 (26.5)**	**20 (26.3)**
Middle (7 - 12 years)	**13 (16.4)**	**18 (23.6)**
Low (≤ 6 years)	**28 (35.4)**	**22 (28.9)**
Illiterate	**17 (21.5)**	**16 (21.0)**
Nationality		
Saudi	**49 (62.0)**	**22 (28.9)**
None-Saudi	**30 (37.9)**	**54 (71.0)**
Residence		
Rural	**41 (51.8)**	**32 (42.1)**
Urban	**38 (48.1)**	**44 (57.8)**
Employment status		
None-employed	**51 (64.5)**	**49 (64.4)**
Employed	**28 (35.4)**	**27 (35.5)**
Clinical symptoms		
Abnormal vaginal discharge	**21 (26.5)**	**NA**
Foul odor	**8 (10.1)**	**NA**
Vulvar itching	**13 (16.4)**	**NA**
Burning sensation	**9 (11.3)**	**NA**
Lower abdominal pain	**14 (17.7)**	**NA**
Dyspareunia	**6 (7.5)**	**NA**
Dysuria	**8 (10.1)**	**NA**

NA - not applicable.

T. vaginalis antigen, were proved as true negatives by the nested PCR. Taking the nested PCR test results as a nominated gold standard, the wet mount microscopy achieved sensitivity, specificity, NPV, PPV of 69.5%, 100%, 94.9%, and 100%, respectively. In contrast, the OSOM Trich exhibited sensitivity, specificity, NPV, PPV of 86.9%, 100%, 97.7% and 100%, respectively, Table 3.

Distribution of infection among women in relation to the sociodemographic characters

Table 4 demonstrates the percentages of *Trichomonas* infections among cases and controls in relation to socio-demographic parameters. It shows that the highest percentage of infections among cases was observed among the age group from 30 to 39 years (35.1%), while the lowest prevalence was among the age group ≥ 40 years (5.8%) and age group 18 - 29 years. This difference was

Table 2. Diagnostic test results of 155 studied women.

Test	Cases		Control		Total		Test of sign.	
	positive n (%)	negative n	positive n (%)	negative n	positive n (%)	negative n	χ^2	p-value
Microscopy	15 (18.9)	64	1 (1.3)	75	16 (10.3)	139	13.06	< 0.001 *
OSOM Trich	15 (18.9)	64	5 (6.5)	71	20 (12.9)	135	5.86	0.015 †
Nested PCR	16 (20.2)	63	7 (9.2)	69	23 (14.8)	132	3.73	0.053

* - highly significant difference.
† - significant difference.

Table 3. Diagnostic performance of microscopy and OSOM Trich versus the nested PCR test's findings.

Test	Positive		Negative		Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	Agreements % (Kappa test)
	true	false	true	false					
Wet mount microscopy ††	16	0	132	7 *	69.5 (47.0 - 86.7)	100 (97.2 - 100.0)	100	94.9 (91.0 - 97.2)	79.1 (0.323) †
OSOM Trich test	20	0	132	3	86.96 (66.4 - 97.22)	100 (97.2 - 100.0)	100	97.7 (93.8 - 99.2)	77.9 (0.342) †
Nested PCR assay	23	0	132	0	NA	NA	NA	NA	NA

CI - 95% confidence intervals, PPV - positive predictive value, NPV - negative predictive value, NA - not applicable.
* - The OSOM Trich and the nested PCR both identified 4 of these samples to be positive, while the PCR alone found 3 of them to be positive.
† - There was fair agreement between the test and nested PCR.
†† - Wet mount microscopy and the OSOM Trich had good agreement (% agreement = 80.43 and kappa = 0.351).

statistically highly significant (χ^2 = 9.57; p = 0.008). It was shown that the difference between symptomatic cases and the asymptomatic controls was statistically significant at the age groups 30 - 39 years (χ^2 = 3.87; p = 0.049). Concerning duration of marriage, the highest burden of infection in cases was seen in women whose marriage lasted between 10 and 30 years (8/13; 61.5%). While the lowest prevalence was seen among cases with < 10 years (10.2%) and > 30 years marriage. This difference was statistically highly significant (χ^2 = 16.85; p < 0.001). Regarding the level of education, the highest percentage of infections among cases was observed among illiterate cases (41.1%), while the lowest burden was exhibited among cases with a high level of education (4.7%), followed by cases with low and middle education levels (17.8% and 23%, respectively). The difference in positivity among cases with different levels of education was statistically significant (χ^2 = 7.89; p = 0.048). It was also shown that the difference between cases and controls was statistically highly significant at illiteracy as the lowest education level (χ^2 = 5.47; p = 0.019). As regards the residence, Table 4 demonstrates that the percentage of infection in cases from urban areas (28.9%; 11/38) was higher than cases from rural places (12.1%; 5/41) when compared to the controls (6.8% and 12.5%, respectively), and this difference was statistically highly significant (χ^2 = 7.05; p = 0.007). In relation to the employment status, the table shows higher infection among employed cases (35.7%) than the non-employed cases (11.7%), compared to the controls (18.5% and 4%, respectively). This difference between cases and control groups was statistically highly significant (χ^2 = 7.05; p = 0.007). Lastly, concerning the nationality, the percentage of infection in non-Saudi cases (23.3%) was higher than in Saudi cases (18.3%) as compared to the controls (5.5% and 18.1%, respectively) and this difference was statistically highly significant (χ^2 = 5.81; p = 0.015).

Distribution of infection among women in relation to the presenting symptoms

Figure 2 shows the frequency variations of symptoms among women who screened positive and negative for *T. vaginalis*. It was shown that the infection was most frequent in women presenting with lower abdominal pain (64.2%; 9/14), followed by women suffering from vulval itching (38.4%; 5/13), abnormal vaginal dis-

Table 4. Characteristics of 16 symptomatic women and 7 asymptomatic women screened positives for *Trichomonas vaginalis.*

Character	Cases (n = 79) % (n/total)	Controls (n = 76) % (n/total)	Test of sign.	
			χ^2	p-value
Demographics				
Age per years				
Median (IQR)	**30 (19 - 46)**	**36 (29 - 42)**	**-**	**-**
18 - 29 years	**8.0 (2/25)**	**6.2 (2/32)**	**0.06**	**0.797**
30 - 39 years	**35.1 (13/37)**	**13.7 (4/29)**	**3.87** †	**0.049**
≥ 40 years	**5.8 (1/17)**	**6.6 (1/15)**	**0.01**	**0.927**
χ^2 (p-value)	**9.57 (0.008)** †	**1.17 (0.554)**		
Duration of marriage				
< 10 years	**10.2 (5/49)**	**5.7 (3/52)**	**0.68**	**0.409**
10 - 30 years	**61.5 (8/13)**	**22.2 (2/9)**	**3.31**	**0.068**
> 30 years	**17.6 (3/17)**	**13.3 (2/15)**	**0.11**	**0.737**
χ^2 (p-value)	**16.85 (< 0.001)** †	**2.86 (0.238)**		
Education level				
High (> 12 years)	**4.7 (1/21)**	**10.0 (2/20)**	**0.04**	**0.519**
Middle (7 - 12 years)	**23.0 (3/13)**	**5.5 (1/18)**	**2.06**	**0.151**
Low (≤ 6 years)	**17.8 (5/28)**	**13.6 (3/22)**	**0.16**	**0 .686**
None-educated	**41.1 (7/17)**	**6.2 (1/16)**	**5.47** †	**0.019**
χ^2 (p-value)	**7.89 (0.048)** †	**0.98 (0.804)**		
Nationality				
Saudi	**18.3 (9/49)**	**18.1 (4/22)**	**0.03**	**0.985**
None-Saudi	**23.3 (7/30)**	**5.5 (3/54)**	**5.81** †	**0.015**
χ^2 (p-value)	**0.28 (0.594)**	**2.98 (0.084)**		
Residency				
Rural	**12.1 (5/41)**	**12.5 (4/32)**	**0.001**	**0.968**
Urban	**28.9 (11/38)**	**6.8 (3/44)**	**7.05** †	**0.007**
χ^2 (p-value)	**3.42 (0.064)**	**0.71 (0.397)**		
Employment status				
None-employed	**11.7 (6/51)**	**4.0 (2/49)**	**2.00**	**0.156**
Employed	**35.7 (10/28)**	**18.5 (5/27)**	**2.04**	**0.152**
χ^2 (p-value)	**6.41 (0.011)** †	**4.33 (0.037)** †		

† **- highly significant at p < 0.05.**

charge (38%; 8/21), dysuria (37.5%; 3/8), odor (25%; 2/8), dyspareunia (16.6%; 1/6), and lastly, in women presenting to the outpatient clinic with a burning sensation (11.1%; 1/9). As shown in Table 5, a highly significant difference was observed regarding infection distribution among cases for the presence of lower abdominal pain and abnormal vaginal discharge (χ^2 = 20.42; p < 0.001 and χ^2 = 5.63; p = 0.017, respectively).

DISCUSSION

This is the first trial carried out in a female population from Taif, Saudi Arabia, to assess the burden of *T. vaginalis* infection and to search for the possible risk factors associated with this infection.

In this study, the *T. vaginalis* infection was identified in ~20% of symptomatic women. *T. vaginalis* is not a reportable infection in many countries including Saudi Arabia. As such, there is a lack of *T. vaginalis* case-reporting data at the regional and the global levels. Infec-

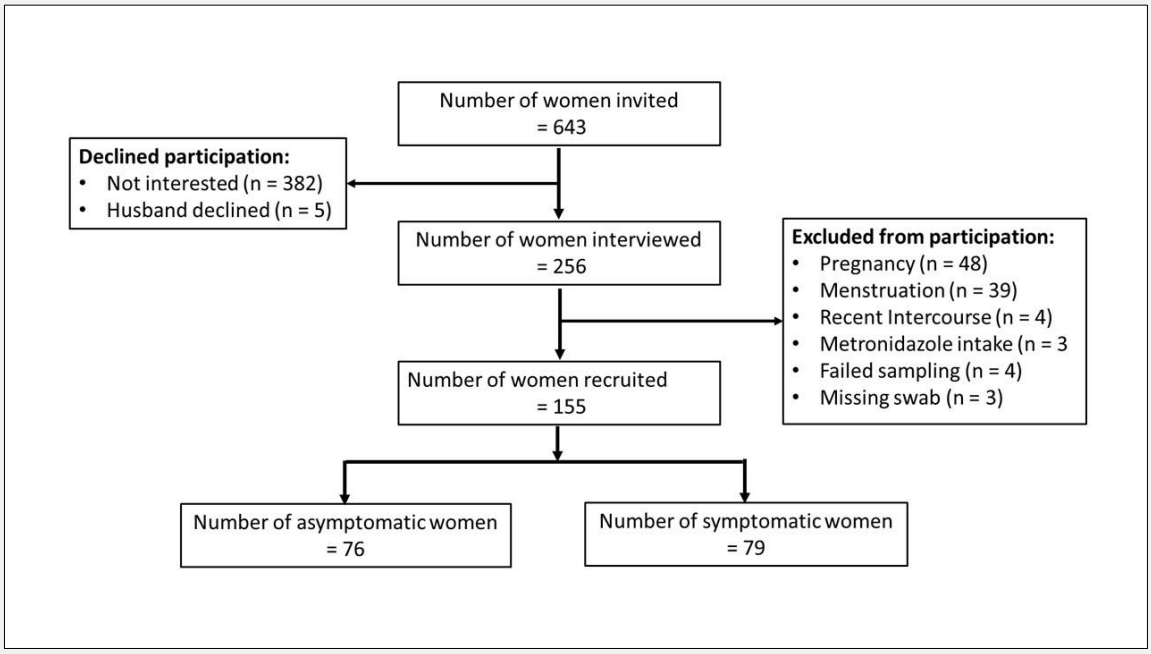

Figure 1. Women's recruitment profile during the period between June 2018 to May 2019.

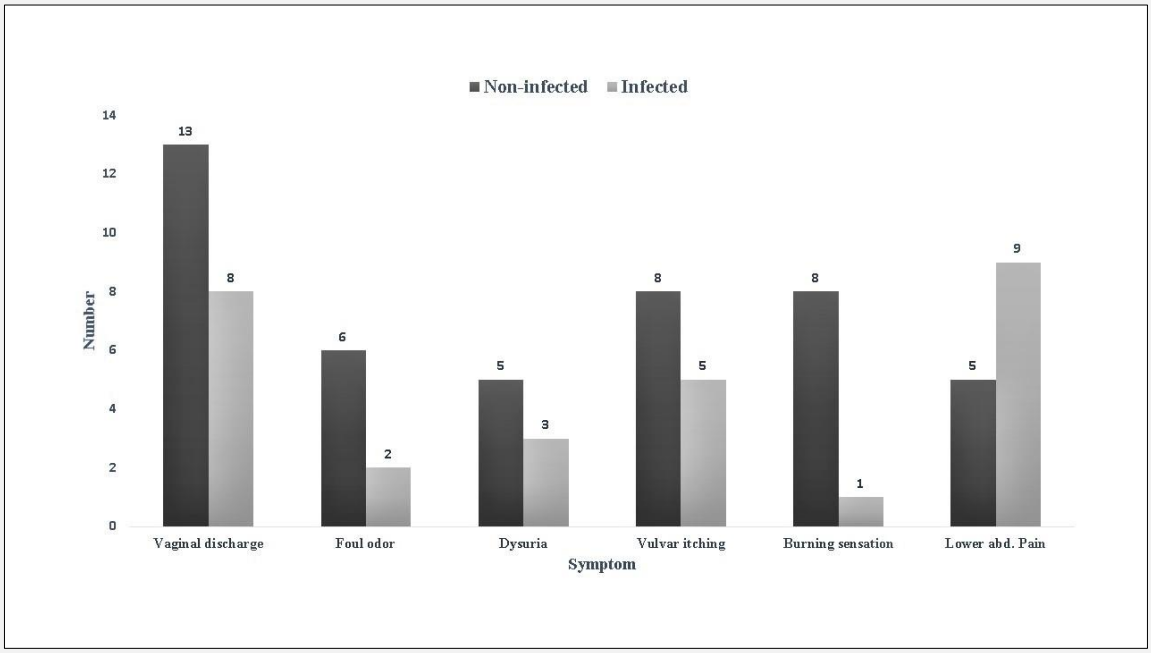

Figure 2. Proportions of symptoms in 79 women screened positive or negative for *Trichomonas vaginalis*.

tion has been detected in other Saudi populations, with rates ranging from 0.7% to ~28%. In Jeddah, the second largest Saudi city, infection at a frequency of 0.7% has been reported in vaginal swabs of women aged 15 - 50 years [13]. In Riyadh, the largest city in Saudi Arabia, the infection has been identified in 4% of hospital-based population relying on culturing the vaginal discharge swabs [14]. Moreover, another study done in Jeddah reported a frequency of ~5% in a female cohort using urine samples [15]. Based on hospital records, a 5-year survey (1995 - 1999) carried out in Jeddah recorded trichomoniasis with an annual incidence of ~20 per 100,000 population reported [7]. The high prevalence observed in the study's population compared to other populations may be due to the combination of different techniques adopted in this study which facilitate the diagnosis of this protozoon. Outside Saudi Arabia, in the United States, a frequency of ~3% was recorded for the infection in one report [16] and a frequency of ~2% in another [17]. In Africa, much higher prevalence rates in women's partners have been reported for trichomoniasis in Africa. A frequency of ~18% was reported in a study performed in southern Ghana [18]. In addition, among women in Los Angeles, California, a frequency of 37% was exhibited [19]. In Islamic societies, much lower infection rates have been recorded, e.g., ~1% in Libya [20], ~2% in Iran [21]. It is important to bear in mind that these prevalence estimates have been reported in populations that differ in their type, size, cultural behavior, and their socio-demographic and clinical characteristics. All these factors could explain the variability of estimates between studies. Also in this study, the *T. vaginalis* infection was detected in ~9% of asymptomatic women. As previously stated, at least 80% of *T. vaginalis* infections are asymptomatic. Asymptomatic infections pose a public health issue. In addition to the possibility of infection transmission to the sex partners, it has been linked to an increase in the risk of having HIV infection [4].

Further in the current study, a nested PCR assay rapid antigen detection test in addition to the wet-film microscopy were validated in symptomatic and asymptomatic women. The PCR exhibited the highest performance followed by the OSOM Trich (~87%) and the wet mount method (~70%). The low sensitivity of the microscope in infection detection has been frequently reported. According to one study, the sensitivity of the routine microscopy ranged between 38% and 82% [22]. The OSOM Trich, a capillary-flow enzyme immunoassay based on trichomonas membrane proteins, can detect *Trichomonas* in 10 minutes [23,24]. Sensitivity of 75% and ~98% have been recorded for the test in populations with a low prevalence of *T. vaginalis* infection [25]. It is worth to verify that variation of sensitivities among studies may be attributed to the infection prevalence and/or the nominated gold standard test. The test shows promise to be used as a home screening tool for trichomoniasis in high-risk women, particularly in highly conservative countries.

In Saudi Arabia and many other Arabic countries, although the *T. vaginalis* infection prevalence measures appear to be increasing, the epidemiological data on the infection remain scarce. Nonetheless, our study has provided a comprehensive and unique insight into the associated risk factors of *T. vaginalis* infection in the studied population. The study revealed that the infection burden peaked significantly among symptomatic women aged 30 - 39 years, non-Saudi citizens (~23%), 10 - 30 years of marriage (~62%), uneducated (~41%), urban residents (~29%), and among those employed (~36%). Coinciding with this finding, other studies conducted in different countries have identified various factors associated with infection in women. Among them are older ages, black ethnicity, a higher number of sexual partners in the preceding year, prostitution, same-sex partners, illegal drug use, no condom use, other sexually transmitted diseases, and low education [26,27]. The reasons for this association are still a matter of speculation. A variety of factors including treatment trends, access to care, social and cultural factors are likely to have contributed to the variation in *T. vaginalis* prevalence in different regions.

Also in our study, the most common symptoms found in women who screened positive for *T. vaginalis* were lower abdominal pain, vulvar itching, abnormal vaginal discharge, dysuria and foul smell, similar to those described by other authors [28]. Although these symptoms may occur in other gynecological infections, these symptoms are strong indications for the clinical diagnosis of trichomoniasis, as confirmed in this study by the higher prevalence of these symptoms among *T. vaginalis*-infected women. Lastly, despite our knowledge of its importance, detection of the other sexually transmitted infections such as *Neisseria gonorrhea* and *Chlamydia trachomatis* and the identification of *T. vaginalis* clinical isolates remained beyond the scope of this study due to time and funding limitations.

In conclusion, *T. vaginalis* infection is prevalent in the women studied, almost half of them were asymptomatic. Identification of a large proportion of asymptomatic infections were missed using the wet-film microscopy. On the contrary, the nested PCR was most sensitive followed by the OSOM Trich rapid test. Women aged 30 - 39 years, urban dwellers, non-educated, and non-Saudi residents were high-risk groups. Our study's results could inform policymakers, physicians, and the general public to improve sexual health and help raise awareness of this curable sexually transmitted infection that is often ignored.

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Declaration of Interest:
The authors have no conflicts of interest to disclose.

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