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Prevalence of trichomonas vaginalis infection and evaluation of diagnostic methods in women attending maternity teaching hospital in Erbil city, Iraq

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Abstract

Background and objective: *Trichomonas vaginalis*, an anaerobic, extracellular flagellated protozoan parasite, widespread sexually transmitted infection (STI). The objectives were to determine the prevalence of *Trichomonas vaginalis* in women attending to Erbil Maternity Teaching Hospital by different methods using microscopy, rapid and culture methods.

Methods: In this cross-sectional study from October of 2024 to June of 2025, 400 high vaginal swab (HVS) samples and urine samples were taken from women attending Maternity Teaching Hospital to detect the prevalence of *Trichomonas vaginalis* infection by different methods such as direct wet mount, rapid and culture methods.

Results: In this study, *T. vaginalis* was detected in 12 cases (3%) by culture and 9 cases (2.25%) by direct wet mount among 400 high vaginal swab samples. Of these, 120 were also tested by rapid diagnostic test, yielding 3 positives (2.5%). From the same patients, 400 urine samples were examined, with 4 positives (1%). Infection occurred only in married women, with the highest prevalence in the 18–29 age group (5.88%). No significant differences were found by age or residence, but significant associations were observed with clinical symptoms, whiff test results, vaginal discharge colour, and vaginal pH.

Conclusion: *Trichomonas vaginalis* infection was significantly linked to elevated vaginal pH and was most common in symptomatic women with pruritus and dyspareunia, particularly in the 18–29 age group. Culture detected more cases than wet mount or rapid tests, supporting the use of multiple diagnostic methods and targeted screening for early intervention.

Keywords: Trichomoniasis, Prevalence, Wet mount, Culture, Erbil.

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Introduction

The flagellated protozoan parasite *Trichomonas vaginalis* (*T. vaginalis*) is the primary cause of trichomoniasis and is found in the human urogenital tract (1). Trichomoniasis is a prevalent non-viral parasitic diseases, spread by sexual contact, that affects people all over the world (2). *T. vaginalis* prevalence can differ according to sociocultural variables, sex, geographic location, and personal health (3). *T. vaginalis* one of the most harmful species which is irritates living tissue, especially epithelial cells, and lyses the mucoid layer of the genital tract. This results in the invasion of nearby cells with specific causative agents, which raises the risk of issues such duct thickness, papilloma formation, and neoplasia. Infected women may present with symptoms including strawberry cervix, pruritus, and a yellowish-green foamy discharge are some of the symptoms that an infected woman may experience (4).

According to data from the World Health Organization (WHO), there were an estimated 156 million cases of trichomoniasis globally, with the most recent prevalence estimates for men and women being 5.3% and 0.6%, respectively (5).

Trichomonas vaginalis infections can result in cervicitis, vaginitis in women, and unfavourable delivery outcomes (6). Furthermore, *T. vaginalis* typically raises the risk of infection with other infections (7).

The prevalence of *T. vaginalis* infection in women of reproductive age and its correlation with severe unfavourable reproductive outcomes highlight the problem's public health significance (8). The fact highlights the problem's significance for public health. Furthermore, this infection may raise the risk of human immunodeficiency virus (HIV) transmission and other sexually transmitted diseases (STDs), which are known to occur very commonly in women. *T. vaginalis* infections are common in women who are fertile and are linked to major complications for reproduction outcomes (9). Chronic genitourinary tract inflammation brought on by infection may potentially result in infertility. According to a number of studies, individuals infected with *T. vaginalis* have unfavourable reproductive consequences. Furthermore, trichomoniasis is linked to the prevalence and severity of prostate cancer and cervical dysplasia (10). Based on a recent meta-analysis on cervical neoplasia, the researchers found a significant positive association between *T. vaginalis* infection and the development of cervical neoplasia (11).

Vaginal pH is crucial for maintaining the vaginal microbiome and protecting against infections like bacterial vaginosis and vulvovaginal candidiasis. A balanced

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pH between 3.5 and 4.5 inhibits pathogenic overgrowth, while changes increase susceptibility to infections and gynaecological complications (12).

Trichomonas vaginalis is classically described as lacking a true cyst stage, existing primarily as a trophozoite that replicates by longitudinal binary fission, inhabiting the urogenital tract, in females, it colonizes the vagina, cervix, and urethra, while in males, it resides in the urethra, prostate, and occasionally the epididymis and transmitted mainly via sexual contact (13).

The present study aimed to reveal the prevalence and associated risk factor of *T. vaginalis* infection among women attending to Maternity Teaching Hospital by different laboratory methods.

Materials and methods

Study population

In this cross-sectional study, a total of 400 high vaginal swab (HVS) samples and urine samples were taken from women attending to Maternity Teaching Hospital in Erbil City. Following the data collection by high vaginal swab three high vaginal specimens were collected from all 400 women ages from 18-65 year using sterile cotton swabs from the posterior fornix, and urine sample from all 400 women attending to Maternity Teaching Hospital department of Pap smear, from of October 2024 till of June 2025.

Inclusion criteria: All age groups of females between 18-65 years old with symptomatic and asymptomatic were included in the current study.

Exclusion criteria: All age groups who were pregnant or presented with contact bleeding were rejected in current study.

Vaginal examination: A gynaecologist examined each woman's vagina for vaginal discharge sterilized Cusco speculum is inserted to visualize the vagina and cervix, and vaginal discharge characteristics such as amount, colour, character, and smell are noted. The vaginal discharge is collected from the lateral walls and posterior fornix using cotton swabs. The pH paper is either touched directly to the vaginal fluid or to the speculum after withdrawal to determine vaginal pH, with a pH above 4.5 suggesting bacterial vaginosis or trichomoniasis (14).

Vaginal swabs: The sterile disposable speculum (Cusco) was introduced into the vagina without any lubrication or solution; after that, three vaginal swabs were taken from women who were in a lithotomy posture fromthe posterior fornix, with the help of assistance of clinicians or specialist physicians.

Methods

Direct wet mount

The first high vaginal swab (HVS) samples were taken with the help of assistance of clinicians or specialist

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female physicians with sterilized cotton wool swabs taken from the vagina's posterior fornix. The first sample was transferred into 1 ml of 0.9% sterile normal saline and mixed thoroughly. A drop of the mixture was placed on a clean microscope slide, covered with a covers lip and observed under light microscope using × 10 objective lens for motile trichomonads, all microscopic analyses were completed within 30 minutes of sample collection, and were confirmed using a × 40 objective lens. Using the vaginal sample wet mount technique, specimens containing *T. vaginalis* trophozoites (one or more motile trichomonads) proved positive (15).

The urine sample was centrifuged at 3500 rpm for 5 minute and the sediment is examined to general urine examination (GUE) to detect trophozoite of parasite.

Culture Method

The second swab was inoculated to newly prepared thioglycolate broth medium and incubated at 37C for 7 days, every day to check for *T. vaginalis* trophozoites under a microscope. Negative specimens were kept in the same conditions until the seventh day following the original inoculation. Then classified samples as negative for *T. vaginalis* by HVS culture technique if they showed no trophozoites under a microscope.

Preparation of thioglycolate broth medium

Modified thioglycolate culture: The medium was used at a concentration of 29.5 g in every one Liter of distilled water. The well mixed medium was sterilized by autoclaving at 121°C for 15 minutes then stored in refrigerator. Just before use the media were taken out of refrigerator and let to take the room temperature and then 10 ml of horse serum, and 2ml of each antibiotic solution Fluconazole 2mg/ml, Penicillin G 1000000 U, and Gentamicin 80 mg/ml were added (16).

After adding antibiotic solution and horse serum we calculate the PH of media by PH meter and adding 1N HCL to bring it down and adjust it to the desired value.

The second vaginal swab was inoculated into thioglycolate culture medium after collection and incubated at 37°C, with daily microscopic examination for the presence of *T.vaginalis* trophozoites. Negative specimens were continually incubated in the same condition until the 7th day after the initial inoculation, after which samples with no trophozoites upon microscopic examination were considered negative for *T. vaginalis* by HVS culture method (15).

Whiff Test

The third swab was performed to whiff test and to detect of vaginal pH. The whiff test, also known as the amine

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test, is a simple bedside diagnostic test used to detect trichomoniasis. It involves mixing vaginal secretions with potassium hydroxide (KOH) to identify the presence of volatile amines, which produce a characteristic fishy odour (17).

Vaginal pH examination

Vaginal discharge was examined for pH by dropping a drop onto a pH testing paper, and the colour shift was used to determine the pH. In some cases were vaginal discharge is too few performed by attaching a short piece of pH paper with aid of forceps and dipping it into the vaginal discharge in the lateral fornices, it was simple to measure the vaginal pH. Then, contrasted with the colours and associated pH values on a reference chart.

Ethical consideration

The study received approval from Hawler Medical University's College of Health Sciences Ethics Committee. Approval was also obtained from the Ministry of Health and the Director of the Teaching Maternity Hospital in Erbil (no. 26140) in 19/9/2024. Additionally, verbal consent was obtained from the participants in this study, who promised that this information would remain strictly confidential and could not be used for purposes other than this study.

Statistical Analysis

After the results were obtained, data analysis was achieved by SPSS v26, the data were presented in the form of percentages, some factors that may have a role in the spread of *T. vaginalis* infection were studied using the Chi-square test, and *P-values* <0.05 were considered statistically significant.

Results

The overall percentage of *T. vaginalis* infection was 3% (12/400) using the culture method. Statistically, no significant differences (P-value = 0.258) were observed in trichomoniasis concerning laboratory tests by Chi-square test, as shown in Table (1).

Table 1. Detection of Trichomonas vaginalis using different laboratory tests

Laboratory tests	No. of Positive (%)	No. of negative (%)	Total	X^2	*P-value*
Wet mount for urine	4 (1)	396 (99)	400	4.024	0.258
Direct wet mount	9 (2.25)	391 (97.75)	400		
Culture	12 (3)	388 (97)	400		
Rapid	3 (2.5)	117 (97.5)	120		

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As illustrated in Table (2), there were no significant differences in trichomoniasis according to age group (P-value = 0.10) and according to residency (P-value = 0.723) using the Chi-square test, in spite of the highest rate of infection being detected in the age group (18-29 years), which was (5.88%), and in urban areas it was (3.77).

Table 2. Prevalence of Trichomonas vaginalis infection among age groups, residency, clinical symptoms and discharge colour using culture method

Factors		No. of Positive (%)	No. of Negative (%)	Total	X^2	*P-value*
Age group	18-29	5 (5.88)	80 (94.12)	85		
	30-39	7 (4.17)	161 (95.83)	168		
	40-49	0 (0)	111 (100)	111	7.758	0.10
	50-59	0 (0)	31 (100)	31		
	60-65	0 (0)	5 (100)	5		
Residency	Rural	10 (2.88)	337 (97.12)	347		
	Urban	2 (3.77)	51 (96.23)	53	0.126	0.723
Clinical symptoms	Asymptomatic	0 (0)	61 (100)	61		
	Pruritus, Dyspareunia	7 (6.61)	99 (93.39)	106		
	Lower abdominal pain, Dysuria	0 (0)	83(100)	83	9.24	0.026*
	Vaginal discharge	5 (3.33)	145(96.67)	150		
Discharge Colour	White to grey	1 (0.48)	208 (99.52)	209		
	Yellow to green	11 (5.75)	180 (94.25)	191	9.563	0.002*
	Total	12 (3)	388 (97)	400		

*Statistically significant

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While we observed significant correlation between T. vaginalis infection and clinical symptoms (P-value = 0.026) and with colour of vaginal discharge (P-value = 0.002) using Chi-square. The commonest clinical symptoms were pruritus and dyspareunia, which had the highest rate of infection (6.61%), and in yellow to green discharge, the highest rate of infection was 11 (5.75%).

Our study confirms a strong association (P-value = 0.001) between elevated vaginal pH and T. vaginalis infection, as shown in Table (3), underscoring the importance of vaginal acidity in maintaining microbial homeostasis, with the highest rate (14.75%) of infection at pH 6.5.

The significant association was obtained between the whiff test and Trichomonas vaginalis infection (P-value = 0.001), as illustrated in Table (4), and the total percentage of infection was reported (6.28%) by this method.

Table 3. Association between Trichomonas vaginalis infection and vaginal pH level

Vaginal PH	No. of Positive (%)	No. of negative (%)	Total	X^2	P-value
3.5	0 (0)	72 (100)	72	36.26	0.001
4	0 (0)	104 (100)	104		
4.5	0 (0)	32 (100)	32		
5	0 (0)	28 (100)	28		
5.5	1(2.78)	35 (97.22)	36		
6	2 (2.99)	65 (97.01)	67		
6.5	9 (14.75)	52 (85.25)	61		
Total	12 (3)	388 (97)	400		

P-value <0.05 statically significant

Table 4. Comparison between whiff test and culture method for detection Trichomonas vaginalis infection

Culture method	No. of positive (%)	No. of negative (%)	Total	X^2	*P-value*
Whiff test					
Positive	12 (6.28)	179 (93.72)	191	13.53	0.001
Negative	0 (0)	209 (100)	209		
Total	12(3)	388 (97)	400		

P-value <0.05 statically significant

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Discussion

Trichomonas vaginalis infections are regarded as the most prevalent non-viral sexually-transmitted disease (STD) and are similar to other STDs in that the prevalence increases with

increased sexual partners, gonorrhoea, other sexually transmitted diseases, and non-use of barrier contraception (9).

Study conducted in Iraq has frequently reported no statistically significant differences in the prevalence of *Trichomonas vaginalis* when comparing various diagnostic methods. This observation may be attributed to overlapping detection capabilities within the studied populations, as well as methodological factors such as sample handling procedures and the technical proficiency of operators (18). Furthermore, limitations in study design and the influence of real-world clinical conditions may contribute to minimizing the anticipated sensitivity differences among these diagnostic approaches (19).

Our findings also showed no statistically significant differences between the diagnostic methods, which is in agreement with a study conducted in Najaf Governorate in 2024 by Al-Hisnaw and Al-Jorany (20). Although that study reported a higher prevalence rate, the differences in detection between methods were not statistically significant, likely due to overlapping confidence intervals and the sample size.

Despite the fact that major participants exhibited the identical signs of infection with the *T.vaginalis* parasite, the prevalence rate of this parasite was extremely low. All of this suggests that, among married women in the city of Erbil, the parasite *T.vaginalis* does not represent an epidemiologically significant health issue, and its agreement with some study that conducted in Iraq.

According to the current study, the culture technique of vaginal discharge revealed that the rate of trichomoniasis infection among women who attended the Maternity Teaching Hospital in Erbil province was 3%. The prevalence of *T.vaginalis* among women in Iraq has already been determined to be comparable. This result aligns closely with previous studies, in Erbil in 2019 by Nouraddin and Alsakee, it was (3.18%) (21). A related molecular and culture-based investigation of vaginal candidiasis among pregnant women in Erbil and Sulaymaniyah similarly combined culture with genotypic methods to characterize genital tract infections in the region (33).

However, when comparing our findings with other studies conducted in Iraq, there is noticeable discrepancy. For example, a study conducted in Najaf found a rate of infection was 10% by Al Hadraawy et al., 2018 (22), whereas in

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Kirkuk, Salih et al., 2022 (23) indicated a prevalence of 8%. similarly research in Duhok by Murad et al., 2024 (24) reported a higher prevalence rate of *T.vaginalis* infection, reaching 9%.

These differences may be attributed to regional variations in socioeconomic status, sexual behaviour, health education, and laboratory diagnostic capabilities. Additionally, the lower prevalence in Erbil may be due to better health education, improved personal hygiene practices, or differences in access to healthcare services. Another possible explanation could be the inclusion criteria; some studies may have focused only on symptomatic women or those at high risk, whereas the present study included a broader population.

About prevalence of *T. vaginalis* among different age group and residency there were no significant differences. In spite of the highest rate of infection was observed between the age group (18-29) years was (5.88%), followed by (4.17%) in the age group (30-39) years, this agreement with the study concocted in Baquba City, Diyala Province, in 2015 by Al-Hussuny (25) The age group with the greatest infection rate (26-30) it was (12.5%), and in Wasit by Rahi and Jaleel in 2022 (26) the age group of 20-30 years had the highest rate of infection (3%).

The elevated rate of *T. vaginalis* in younger women may be more susceptible due to cervical ectopy**,** which is more common in this age group and provides an environment conducive to infection higher risk is also linked to behavioural characteristics such irregular condom usage, many sexual partners, and early sexual debut (9).

In a study by Taher and co-workers in 2018 (27) that women 25-35 years had significantly higher prevalence (20.3%) than other age associated with older age compared to those aged 20-25 years and this was disagreement for our study that younger age had high prevalence infection.

Also, there were no significant differences observed among the residency of women that had trichomoniasis, that aligns with a study done in Wasit by Rahi and Jaleel, in 2022 (26). But in a study conducted in Duhok by Murad et al, 2024 (24), there was disagreement with our result that rural infection was more than urban. In many studies, differences by residency were small or inconsistent; leading to non-significant statistical results, indicating that residency alone is not a strong independent risk factor.

In the present study, several clinical symptoms were found to be statistically significant in association with *T.vaginalis* infection. Among these, pruritus and dyspareunia, which had the highest range of infection (6.61%) followed by vaginal discharge (3.33%) that showed a strong correlation ($P < 0.05$), indicating

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their potential diagnostic value in clinical settings.

This result aligns with the study conducted in Basra by Kadhum et al., 2020 (28) that pruritus symptom was (5%) and vaginal discharge (10%) and had statistical significance. While in other study conducted in Mosul by Al-Taei, 2022 (29) dyspareunia had a rate of (6.8%), followed by lower abdominal pain with a percentage of (4.9%), and there was no statistically significant difference among clinical symptoms that showed discrepancy with our study. *T. vaginalis* are statistically significant across studies and can be explained by several factors related to study design, population, diagnostic methods, and statistical power. Some studies may include more symptomatic individuals (e.g., clinic attendees), while others may include a general population with a high proportion of asymptomatic carriers.

In this study, we observed a statistically significant association between the colour of vaginal discharge and *T.vaginalis* infection. Among infected participants, yellow to green discharge was reported more frequently compared to white to grey discharge, were the rate of infection was (5.75%) and (0.48%) respectively (*P-value* <0.05), this finding was in strong agreement with previous study by Meena and Bansal in 2016 that reported yellow to green discharge had high rate of infection (14.2%), whereas white to green was (0%) (30).

The infection rate of *T. vaginalis* according to discharge colour is not the same across studies due to differences in parasite load, co-infections, diagnostic methods, and population characteristics (9).

Our findings indicate a strong association between elevated vaginal pH and the presence of *T.vaginalis.* Women with vaginal pH>6.5 showed a significantly higher infection rate (14.75%), followed (2.9%) at pH 6, and then (2.7%) at pH 5.5. This aligns with the known biology of *Trichomonas vaginalis*, which thrives in a less acidic environment (9), similar results were reported by Kadhum et al., 2020 (28) the higher infection rate in pH 6-6.5 (50%), pH-5.5 (40%) who found a threefold increased risk of infection at pH levels above 5. These results highlight the potential role of vaginal pH as a risk factor and suggest its utility in guiding clinical suspicion and diagnosis.

The whiff test is a commonly used clinical tool for detecting abnormal vaginal discharge, particularly in bacterial vaginosis. However, positive results may also be observed in cases of *T. vaginalis* infection due to similar alterations in the vaginal microbiome. In our study, we found a higher prevalence of *T. vaginalis* infection among women who tested positive in the whiff test (6.28%) compared to those with

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negative results (P-value <0.01). This suggests that a positive whiff test may serve as an additional indicator for *T. vaginalis* infection. Similar indicator in a study performed by Von Glehn and its co-worker in 2017 (31) reported that 20% of patients had a positive whiff test, this was in agreement with our result, The whiff test in *T. vaginalis* infection has low sensitivity (~52%) and moderate specificity (~79%) when used alone, meaning it misses many true infections but is fairly good at ruling in infection when positive (32).

Overall, the prevalence rate observed in this study disagrees with most other Iraqi studies that have reported higher rates of *T. vaginalis* infection. These differences underscore the importance of local epidemiological surveillance and tailored public health interventions based on regional data.

This study has several limitations. It was conducted at a single center, which may limit the generalizability of the findings to the broader population. The sample population consisted solely of women attending a maternity hospital, potentially introducing selection bias and under representing asymptomatic or unmarried individuals. Furthermore, due to limited resources, highly sensitive molecular diagnostic techniques such as polymerase chain reaction (PCR) were not employed, which may have resulted in underestimation of the actual prevalence.

Despite these limitations, the findings emphasize the value of combining diagnostic methods to improve detection of *T. vaginalis* and underscore the importance of routine screening in symptomatic women. Future research using multi-center designs and molecular diagnostics is recommended to validate and expand upon these findings.

Conclusion

Our study found a significant association between *T. vaginalis* infection and elevated vaginal pH, with the highest infection rates observed among symptomatic women, particularly those experiencing pruritus and dyspareunia (6.61%), and in the 18–29 age group (5.88%). Culture detected more positive cases than direct wet mount and rapid testing within the respective sample sets examined; however, the true sensitivity of these methods could not be determined, as they were not all performed on the same set of specimens. These findings highlight the importance of employing multiple diagnostic approaches to improve detection rates and support the need for targeted screening among symptomatic women. For more robust diagnosis and surveillance, future studies should incorporate advanced molecular techniques, such as PCR alongside conventional methods, and

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apply all tests to the same sample set to enable accurate evaluation of diagnostic performance.

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Competing interest

The authors declare that they have no competing interests.

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