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Molecular diagnostic of *Trichomonas Vaginalis* isolated from woman's in honeymoon

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Abstract
Background: Throughout the initial phases of marriage, commonly known as the "honeymoon" phase, women may develop severe symptoms of *trichomoniasis*, a sexually transmitted illness caused by the prevalent protozoan parasite, *Trichomoniasis.virginals.*
Aim of study: To emphasize the significance of rapid detection of *T. virginis* in honeymoon ladies, the present study used vaginal sweep specimens and Real-Time PCR to identify the prevalence of T*. virginis* among these women.
Methodology: A total of 140 vaginal swab honeymoon women aged 14-37 years were enrolled y were collected from women attending gynecological clinics during their honeymoon period. Detection of T. vaginalis DNA was performed using real-time (q PCR) technique.
Results: The results a revealed T. vaginalis prevalence of 6.4%. No significant differences were observed in the mean age or residence (urban vs. rural) between *T. Vaginalis* positive and negative women.
Conclusion: qPCR effectively identified Trichomonas vaginalis infections in honeymooned women with vaginitis. Marital duration and age (14-37 years) significantly influenced pathogen prevalence, underscoring the role of behavioral and hormonal factors in the etiology of vaginitis.

Keywords: *Trichomonas. Vaginalis*, vaginal swab honeymoon women & real-time

Introduction

Trichomonas.vaginalis, a flagellate protozoan, is the only natural host of *trichomoniasis*, a highly common non-viral, sexually transmitted disease (S.T.D) worldwide. [1]. Many women in the general population suffer from *trichomoniasis* annually worldwide [2]. Women on their honeymoons constitute a distinct representative demographic at elevated risk owing to increased sexual activity, alterations in the vaginal microbiome, and potential exposure to novel pathogens. Severe vaginal infection, which frequently occurs after sexual activity, is also known as honeymoon vaginitis. In many cases, mild honeymoon vaginitis resolves on their own within few days. However, honeymoon vaginitis requires further investigation. [3]. Understanding the influence of specific sexual practices. Given that *T. vaginalis* can ingest Mycoplasma and other bacteria and viruses, several studies have hypothesized that *T. vaginalis* might operate as a "vector" for the introduction of other diseases into the genitourinary tract [4]. In addition, when the parasite attaches to the cervix, vagina, and urethral mucosa, T. vaginalis may trigger local inflammation in the pathophysiology of *trichomoniasis*. The release of a cell-detaching agent called cysteine proteinase causes structural integrity of the urogenital tract and defends the barrier to break down, causing epithelial cells to exfoliate [5]. *Trichomoniasis vaginosis* is caused by the parasite, *Trichomonas vaginalis*. Clinical symptoms include greenish-yellow frothy discharge, purulent discharge, urinary burning, and strawberry-shaped cervix, which is considered one of the causes of premature delivery and rupture of the amniotic sac and cervical and endometrial infections following surgery [6]. Clinically, *trichomoniasis* is associated with severe fertility issues such as ectopic gestation, vaginal inflammation, pre mature breakdown of membranes, premature delivery, low birth weight, and infertility [7]. This study aimed to investigate the prevalence and molecular characteristics of *Trichomonas vaginalis* in women with urinary vaginitis during the honeymoon period. This study also aimed to quantify the presence of these pathogens using quantitative real-time PCR (qPCR) to improve the early diagnosis and enhance the clinical management of vaginal infections in newly married women.

Methodology

A-Patients and study designs

During cross-sectional research has been conducted on 140 women with honeymoon, with age range 14-37 years old, were suffered from urinary vaginitis where admitted to Al-Shamiya General Hospital Also Maternity& Children's Teaching Hospital in Al-Diwaniyah province in a period from the first of October2024 to the end of March 2025. The inclusion criteria were symptomatic women (e.g., dysuria, urgency, frequency, and vaginal discharge) reporting recent sexual activity onset or change. Exclusion criteria typically involved recent antibiotic/antifungal use (within 4 weeks), pregnancy, and menstruation at the time of sampling, which should be explicitly stated. The patients underwent direct interviews using an anonymous questionnaire specifically developed for this study to collect demographic and clinical data, including age, residence, and duration of marriage/sexual activity. A standardized clinical examination was performed and vaginal swab samples were collected from the posterior fornix by a specialist physician using sterile Dacron or rayon-tipped swabs. Swabs were immediately placed into a specific transport medium (Amies, Stuart's) within sterile disposable containers to maintain viability and avoid contamination and were clearly labeled with unique participant identifiers. The samples were transported under appropriate conditions (e.g., ambient temperature with minimal delay or using a cool pack if transported to >30 laboratories for processing).

B-Laboratory Processing and qPCR Analysis

All vaginal swabs were processed within one hour of arrival at the laboratory. Samples that could not be immediately processed were refrigerated at 4°C for a specified maximum period (<24 h) until analysis. Genomic DNA was extracted from each swab sample using a commercial extraction kit (Presto™ Mini gDNA Bacteria Kit, Macro gen Company, Korea), according to the manufacturer's protocol, including appropriate negative extraction controls. DNA concentration and purity were assessed by spectrophotometry (Nano Drop, Specialty Instruments). Quantitative Real-Time PCR (qPCR) assays were performed on a [Specify qPCR Instrument (Applied Biosystems 7500, Bio-Rad CFX96) to detect and quantify copy numbers of key *Trichomonas vaginalis*. targeting their ribosomal RNA genes. Primers were designed for this study using the NCBI Gen Bank database and the Primer3 online software.

Primer sequences (Table (1) and the expected amplicon sizes are reported

Primers were synthesized by Macrogen, Inc. (Seoul, Korea). The qPCR reaction mixture (specified total volume, e.g., 20 µL) contained [Specify Master Mix, for example, 10 µl of 2X SYBR Green Master Mix (Roche)], optimized concentrations of each primer (e.g., 0.5 µM each), template DNA (specified volume/amount, e.g., 2 µL), and nuclease-free water. The optimized thermal cycling conditions were as follows: initial denaturation at 95°C for 3 min, followed by 40 cycles of denaturation at 95°C for 10 s, annealing at 60°C for 30 s, fluorescence data acquisition (Scan) at 60°C for 0.5 sec (for SYBR Green - specific dye used if different), and a final melt curve analysis cycle from 65°C to 95°C (specify increment, e.g., 0.5°C per step) to confirm amplicon specificity. Standard curves for absolute quantification were generated using serial dilutions of known concentrations of target gene plasmids or gDNA. Each run included appropriate negative (no-template control [NTC]) and positive controls for each target.

Table 1: PCR detection gene primers with their nucleotide sequence

Trichomonas vaginalis	F	TTTCCACCGTACCGAAACCTAG	105	X943596.1
	R	ACGGGCGTTTTAACTGCAAC		

C-Quantitative Real-Time P.C.R (q.PCR)

Identification and copy number quantification of bacterial vaginosis from vaginal swab samples were performed using the polymerase chain reaction (qPCR) method. This experiment was performed using a DNA Extraction kit (Presto™ Mini gDNA bBacteria Kit Geneaid, Taiwan).

D-Ethical management of the study

As the present study did not incorporate certain biologically restricted drugs, animals, or genetically altered organisms, it was conducted in compliance with the guidelines and recommendations provided by the College of Medicine, University of Al-Qadisiyah, and Health Ministry. All isolates used in this study were collected without the need for additional material.

Statistical analysis E: Statistical analyses were performed using SPSS version 26 (IBM Corp., Armonk, NY, USA) and Microsoft Excel 2010 software. Descriptive statistics,

Results

The present study collected samples from 140 women aged 14-37 years with honeymoons. Genetic biology-based technological advances are now widely utilized to overcome the challenge of microscopic identification and benefit from the sensitivity, specificity, and accessibility of the technique. PCR is going to begin as the gold standard by which alternative diagnostic techniques, such as microscopy and antibody identification, can be evaluated. The present results show 9 (6.4%) of participants was positive of real-Time PCR in the diagnosis of *Trichomonas vaginalis* parasite out of 140 women with honeymoon, as shown in figure [1]. The agarose gel electrophoresis image showing the PCR product of *Trichomonas vaginalis*.as is shown in Figure (.2).

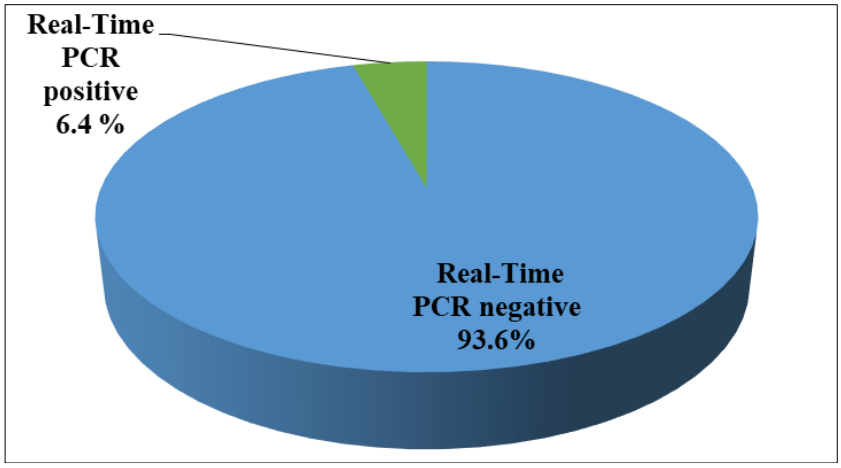

Fig 1: Pie chart displaying the real-time qPCR positive rate of identification for *Trichomoniasis virginals* parasite diagnostic

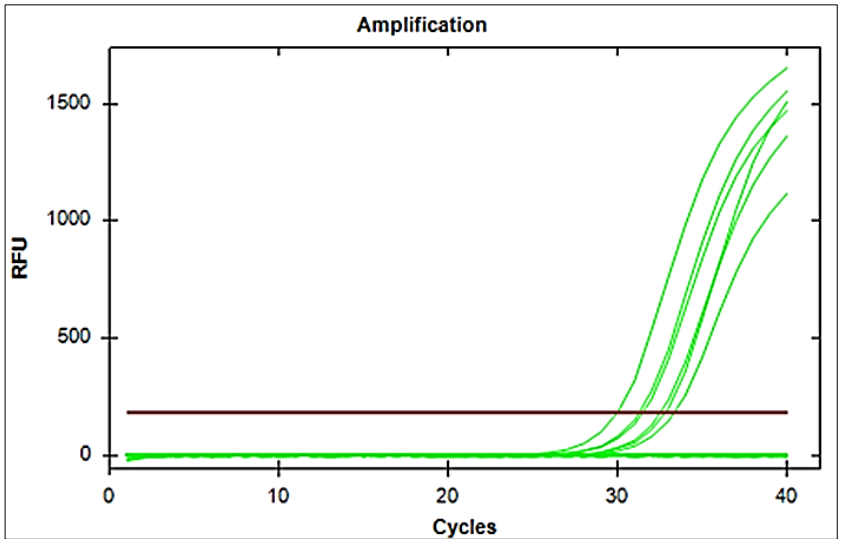

Fig 2: qPCR plot showing amplification of the 18SrRNA gene to detect *Trichomonas vaginalis*.

The average ages as well as residence the allocation are compared based on the findings of a real-time (qPCR) test for the parasite *Trichomonas.vaginalis*.as shown of table 2 According to the current research, patients with positive *T.vaginalis* as well as those with negative *T. vaginalis* had non-significantly different mean ages, suggesting that older people are more susceptible to infection from this parasite than younger people. There was no discernible difference among rural and urban residency within the present research, which suggests that as rural areas become more urbanized, the frequency of infections decreases. Additionally, as urban areas undergo less development in terms of water and sanitation, their infection rates increase to levels equivalent to those of rural areas.

Table 2: Analysis of average ages and frequency of residences based on the findings of a real-time qPCR test for the parasite *Trichomonas vaginalis*

Characteristic	Total n=140	Positive:n=9	Negative: n=131	P
Age (years)				
Mean ±SD	22.21 ±4.69	25.33 ±6.15	22.07 ±4.40	0.096 I NS
Range	14 -37	18-37	14-34	
Residency				
Urban, *n* (%)	86 (61.4%)	6 (66.7%)	80 (61.1%)	0.788 C NS
Rural, *n* (%)	54 (38.6%)	3 (33.3%)	51 (38.9%)	

N: number*of c* ases; **S.d:** standard deviation; **I:** independent*s* amples t-test; **C:** Chi*s*-quare test; **N.S:** not significant at P > 0.05; **S:** Significant at. *P*<0.05.

The frequencies of vaginitis cases based on the findings of a real-time qPCR test for the parasite *Trichomonas vaginalis* are shown in Table (3). According to the current investigation, patients positive *T. vaginalis* and those negative *T. vaginalis* did not significantly vary in the frequency of vaginitis cases. Nonetheless, six (66.7%) of the individuals in the current investigation who tested positive for *T. vaginalis* also had a history of vaginitis.

Table 3: Comparative analysis of the number of vaginitis cases based on the findings of a real-time qPCR test for the parasite *Trichomonas vaginalis*

Characteristic	Total: n=140	Positive: n=9	Negative: n=131	P
Vaginitis Number				
One Time, *n*(%).	108 (77.1%)	6 (66.7%)	102 (77.9%)	0.533 C NS
Recurrent, *n* (%)	32 (22.9%)	3 (33.3%)	29 (22.1%)	

n: Number of cases; **C:** Chi-square test; **N.S**: not Not Significant at $p > 0.05$.

A comparison of the average age and frequency of residence based on the findings of a real-time (qPCR) test for the parasite *Trichomonas vaginalis* is shown in Table (2). The present study showed that there was a non-significant difference in mean age between patients with positive *T. vaginalis* and those with negative *T. vaginalis* indicating that this parasite can infect older patients more than younger patients. In the current study, there was no significant variation between rural and urban residency, which indicates that urbanization of rural areas leads to lower incidence in rural areas, and there is less effort to develop sanitation and water supply areas in urban areas, leading to a higher infection rate in urban areas than in rural areas.

The frequency distribution of married duration according to the results of the Real-Time PCR examination for *Trichomonas vaginalis* parasites is shown in Table (4). The present study showed no significant difference in marital duration between patients positive *T. vaginalis* and those negative *T vaginalis*. However, most patients with positive T. vaginalis in the present study have more than three months married duration, seven (77.8%).

Table 4: Comparison of married duration according to results of real-Time qPCR examination for *Trichomonas vaginalis* parasite

Characteristic	Total: n=140	Positive: n=9	Negative: n=131	P
Married duration				
< 3 months, *n* (%)	70 (50.0%)	2 (22.2%)	68 (51.9%)	0.084 C NS
≥ 3 months, *n* (%)	70 (50.0%)	7 (77.8%)	63 (48.1%)	

N: Number of cases; **C:** Chi-square test; **N.S**: not significant at $p > 0.05$.

Dissuasion

The findings of this study highlight the effectiveness of real-time qPCR in diagnosing *Trichomonas vaginalis* infections among women during the honeymoon period. Out Of 140 participants, 6.4% tested positive for *T. vaginalis* using real-time qPCR, underscoring the technique's sensitivity and specificity compared to traditional methods like microscopy. This aligns with previous research demonstrating that molecular techniques, particularly PCR, are superior for detecting *T. vaginalis*, especially in asymptomatic or low-parasite load cases [8]. The low positivity rate in this study may reflect improved hygiene practices or the demographic characteristics of the sample, but it also emphasizes the need for highly sensitive diagnostic tools to avoid underestimating the prevalence [9]. This study's focus on honeymooned women, a group often underrepresented in *T. vaginalis* research, adds novelty, as this period may involve increased sexual activity, a known risk factor for *trichomoniasis* [10]. The use of real-time PCR as a gold standard in this study is justified by its ability to overcome limitations of microscopy, such as low sensitivity (50-60%) and reliance on immediate sample processing [11]. Technique automation and reproducibility further enhance its utility in clinical and research settings [12]. However, the study design and relatively small sample size may limit the generalizability, suggesting the need for multicenter studies with larger cohorts. Future research could explore correlations between *T. vaginalis* and other sexually transmitted infections (STIs) in honeymooning women, as co-infections may exacerbate reproductive health outcomes [13]. The average number of *T. vaginalis* propagated in the Iraqi municipalities was recorded by Hansh in 2024. The provinces of Maysan as well as Tikrit had the highest incidences of infection at 75% and 62%, respectively, whereas the provinces of Basra as well as Sulaimania had the least expensive getting infected levels at 1.6% and 1.66%, Accordingly, the investigator ascribed this discrepancy to variations in the length of the investigation, sampling size, diagnostic technique, geographical location, and type of social customs that are prevalent in the community. The mean age of all research participants was 22.21 ± 4.69. This parasite can infect older participants, which supports research showing that it is prevalent in all age categories. The present research found a non-statistically significant variation (P = 0.096) in the age average between participants with *T. vaginalis* positive (25.33 ±6.15) and those with *T. vaginal*is negative (22.07 ±4.40). [14]. A study conducted by some researchers confirmed that the infection rate was lower in younger age groups than in older age groups, and the peak of infections was more than 25 years of age [15]. Another study showed that females between the ages of 14 and 20 years suffered less *T. vaginalis* aggression than older females, and the prevalence rate among those between the ages of 14 and 20 years was unstable and had not been previously reported [16]. However, the slightly higher mean age in positive cases could indicate behavioral or biological factors such as prolonged sexual activity or hormonal changes, warranting further investigation [17]. According to residence, the present findings showed there was no significant variation between rural and urban residency, although the infection rate in urban areas to be higher than rural areas (66.7% urban vs. 33.3% rural, p = 0.788), this consistent with [18] of Kirkuk and [19] in Diyala, who reported that metropolitan areas had the highest incidence. Although the majority of Iraqi research found that the prevalence was greater in rural than in urban regions, as [20] in, [21] in Basra, who stated that rural regions had the highest frequency, this contradicts earlier

research that connected greater incidence to remote regions because of the restricted availability of hospitals [22]. The current findings may reflect the impact of urbanization, where rural areas adopt urban lifestyles, thereby reducing disparities in infection rates [23]. Additionally, the comparable sanitation and water supply conditions between urban and rural settings in the study population could explain this uniformity [24]. The absence of a statistically significant disparity between urban and rural residences is intriguing and warrants further exploration through extensive research. This study reinforces the role of qPCR in the accurate diagnosis of T. vaginalis infections, particularly in populations with potential underreporting. The absence of age- or residency-related disparities challenges traditional assumptions and urges the development of updated public health strategies. Addressing limitations through expanded studies and integrating PCR into routine screening could improve *trichomoniasis* management, especially in high-risk groups such as honeymoon women. The study examined the frequency of vaginitis episodes in women diagnosed with *Trichomonas vaginalis* using real-time (q PCR), revealing no significant difference in vaginitis occurrence between PCR-positive and PCR-negative cases ($p = 0.533$) Among T. vaginalis-positive participants, 66.7% experienced a single episode of vaginitis, while 33.3% reported recurrent infections. This distribution closely mirrored. The high proportion of single-episode vaginitis across both groups (77.1% overall) may indicate effective self-resolution or prompt treatment, particularly in honeymooned women who might seek early medical care due to symptom awareness [25]. Notably, the absence of a significant association ($p > 0.05$) between *T. vaginalis* and recurrent vaginitis challenges the clinical assumption that *trichomoniasis* frequently causes chronic inflammation [26]. This discrepancy could stem from the study's demographic homogeneity (honeymooning women) or the high sensitivity of real-time PCR, which detects low-parasite-load infections that may not provoke recurrent symptoms [27]. Further research stratifying participants by parasite load, concurrent STIs, and vaginal microbiota composition (e.g., Lactobacillus depletion) could clarify these relationships [28]. These results underscore the need for comprehensive diagnostic approaches for recurrent vaginitis, as *T. vaginalis* may not always be the primary culprit. Integrating molecular testing with microbiota analysis (e.g., 16S rRNA sequencing) can identify co-factors, such as *Gardnerella vaginalis* or aerobic bacteria [29]. Public health efforts should emphasize differential diagnosis and partner treatment to mitigate the risks [30]. The present study analyzed marriage duration among women with and without *T. vaginalis* infection and showed 7 (77.8%) of PCR-positive cases had been married for more than 3 months compared to 2 (22.2%) with less than 3 months' duration, but this difference was not reach statistically significant ($p=0.084$). This trend suggests that married women longer than three months may be at a higher risk for *trichomoniasis*, possibly due to increased sexual activity during the early marital period or exposure to new sexual partners. The higher prevalence in newlyweds aligns with the established literature on sexual behavior patterns in early marriages. Studies have shown that the frequency of sexual intercourse typically peaks during the first months of marriage, potentially increasing exposure to STIs [31]. Furthermore, the honeymoon period often represents the time of sexual exploration and may include less consistent condom use [32], which could contribute to higher transmission rates. This insignificant difference may be due to the relatively small number of positive cases (n=9) in the present study. Larger cohort studies are required to confirm these findings. Interestingly, the findings are consistent with previous research suggesting that longer-term relationships might show higher STI prevalence due to the decreased condom importance of considering the unique behavioral and biological factors present in early marriages over time [33]. However, our results emphasize the importance of considering the unique behavioral and biological factors present in early marriage biological factors present in early marriage. The intense sexual activity characteristic of the honeymoon period may outweigh the protective effects of monogamy that typically develops in long-term relationships. These results have important implications for sexual health education and screening programmes. They suggested that recently married women, particularly those in the first three months of marriage, may benefit from targeted STI screening and education about safe sexual practices. This approach could be particularly valuable in cultures where premarital sexual activity is uncommon and marital sex represents the primary risk period for STI acquisition [34]. Future research should explore this association using larger samples across different cultural contexts.

Conclusion

The study concluded into the following

This study confirmed that Real Time q PCR is a highly sensitive and specific diagnostic tool for *Trichomonas. vaginalis* infections that showed no significant variation by age or urban/rural residency, suggesting that behavioral and hormonal factors may be more influential in infection risk than demographic variables and that the honeymoon period often involves changes in sexual activity, which may disturb the vaginal microbiome.

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Conflict of Interest

The authors declare no conflict of interest

Data availability

Completely obtained information was involved in this training.

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