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Molecular detection of *Trichomonas vaginalis* from women in Babylon province, Iraq

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Trichomonas vaginalis is one of the most important non-viral microorganisms that cause sexually transmitted infections with infection correlation with HIV that needs continuous surveillance. Our study was based on a collection of 100 vaginal swabs. These samples were tested using microscopy, measuring trophozoites, using Giemsa dye to diagnose *T. vaginalis* and also using PCR that targeted the 18S rRNA gene of the protozoan. The results revealed the presence of the parasite in 64 (64%) of the swabs taken as detected by the PCR. However, this parasite was found microscopically in only 7% of vaginal swab samples. In terms of geographical correlation, the findings demonstrated a significant connection between the prevalence rate and the sample collection regions, in which the highest rates of infection were in women from rural areas at 65.7% while in urban areas it was 60%. Regarding the age of the women infected, the highest prevalence 93.3% was found in the age group 26–35 years, compared to 50% in the 20–24 group and 53.3% in the older age group 36–40 years. The findings recorded higher prevalence rates (65%) detected in women from rural districts compared to urban areas (60%).

Keywords: trichomoniasis; venereal disease; women's reproductive organs; sexually transmitted infections.

Introduction

Trichomonas vaginalis is an anaerobic parasitic protozoan. It was first isolated from vaginal secretion by Alfred François Donné in 1836. *Trichomonas vaginalis* is considered as a mild sexually transmitted disease in humans (Menezes et al., 2016). *Trichomonas vaginalis* does not have mitochondria and it includes a large molecular weight genome of 176 Mbp. This genome constitutes six chromosomes, which characterize this parasite (Carlton et al., 2007). *Trichomonas vaginalis* is a pear-shaped or an oval trichomonad, having four pairs of anterior flagella, an undulating membrane, an axostyle, Golgi apparatus, and unique iron-containing granules in the cytoplasm (Al-Mugdadi et al., 2022). Several ideas about the physiological function of acid phosphatases or mixed phosphatases in *T. vaginalis* have been discussed and experimentally examined, and the results were summarized in a review article by Jeon and in a book chapter by Knecht. However, this paper discusses the following aspects of the location of acid and mixed phosphatases and the ultrastructure of sites used for localization of the enzymes: morphology and location of acid phosphatase in *T. vaginalis*, the whole trichomonad, the pellicle, the hydrogenosomal funnel, the Golgi apparatus, and the granules (Hsu et al., 2020).

Trichomonas vaginalis trophozoite has a pear-shaped or an oval body. It has an anterior and a posterior nucleus (Rigo et al., 2022). The anterior nucleus is horseshoe-shaped and the posterior nucleus is round-shaped. The Golgi apparatus consists of 10 to 12 stacks of cisternae (Maritz et al., 2014). The whole apparatus is oval-shaped and located on the bar-shaped base of pelta. The mitochondrion is generally described as zygote-shaped (Soper, 2004; Sutcliffe et al., 2012; Zhang et al., 2014; Wisner, 2021; Zimmann et al., 2022).

The latest update on the worldwide prevalence provides a rough estimation of more than 100 million cases, increasing to 180 million infections in 2018. A recently published meta-analysis estimated global prevalence in the general population to be 5% with regional prevalence rates ranging from 2–18% in sub-Saharan African countries, 1.1–12.2% in Middle Eastern countries, to an average of 4.7% in Central and Latin

American countries (Lewis et al., 2021). Notably lower prevalence rates of 1.3–3.7% were reported on the Indian subcontinent and 0.6–3.7% in Eastern European countries. In European countries, the prevalence of *T. vaginalis* ranged from 0.5% in Spain, increasing to 1% in Swiss women, 2–8% in women from Ireland, Czechia, and the Slovak Republic. Besides, the prevalence of *T. vaginalis* ranged from 0.4% in Australian women to 2–25% in U.S. women and up to 1 in 10 infected with *T. vaginalis* in Ugandan women (Mirzadeh et al., 2021).

Trichomonas vaginalis is one of the most prevalent parasitic infections worldwide. Recently, WHO has listed this pathogen for further assessments and research as there is little understanding of the global prevalence (Beyhan, 2021). According to estimations, 276.4 million cases of trichomoniasis were reported in 2017, which was about a one-third increase from 2008. Moreover, it affects men and women regardless of socioeconomic status and race. Trichomoniasis remains asymptomatic in most men, which makes it difficult to treat and diagnose. The main site of this pathogen in women is the vagina, which enables it to be transmitted sexually. After chlamydial infection, trichomoniasis is the most widespread sexually transmitted disease globally (Mabaso & Abbai, 2021). There is an elevated incidence and prevalence rate, in addition to an increased resistance profile to the standard treatment. Serious health complications were also reported, which highlights the importance of this infection (Secor et al., 2014). However, asymptomatic *T. vaginalis* cases are another major type of this infection, which represents a challenge in measuring the rates of infection, and making early diagnosis as well as treatment (Soper, 2004; Bachmann et al., 2011; Alderete & Chan, 2023).

Material and methods

A total of 100 vaginal swab samples from women within Babylon province, including Al Qassime, Al-Hilla, and Massayab districts, were collected and examined in the period from September 2023 to January 2024. The samples were maintained using a transport media of 3–5 mL of normal saline and were first examined using traditional microscopic me-

thods. A small quantity of each vaginal swab was taken and placed on a slide. The swab was distributed and dried. Then, immersion in methyl alcohol for 1 minute was done and followed by placing it in Giemsa stain solution for 15 minutes. After that, the washing, draining, and drying steps were performed. Lastly, the slide was examined under a microscope using immersion.

The samples were then subjected to PCR techniques. The trophozoite of the *Trichomonas* parasite is characterized by rapid decomposition, which creates difficulties in microscopic examination. For this reason we also used PCR, which is highly sensitive and specific for the detection of

Trichomonas vaginalis DNA in vaginal swabs.

Results

The findings revealed that trichomoniasis was identified in 7% of vaginal swab samples. These trophozoites were ovoid or pearl in shape ($3-7 \times 1-5 \mu\text{m}$, Fig. 1). The samples were subjected to Genaid DNA purification kit according to instructions, in which 0.5 mL vaginal swab was placed in a 1.5 mL centrifuge tube, which was followed by the procedure of the kit. The extracted DNA was NanoDrop measured. The complete PCR procedures are mentioned in Tables 1, 2.

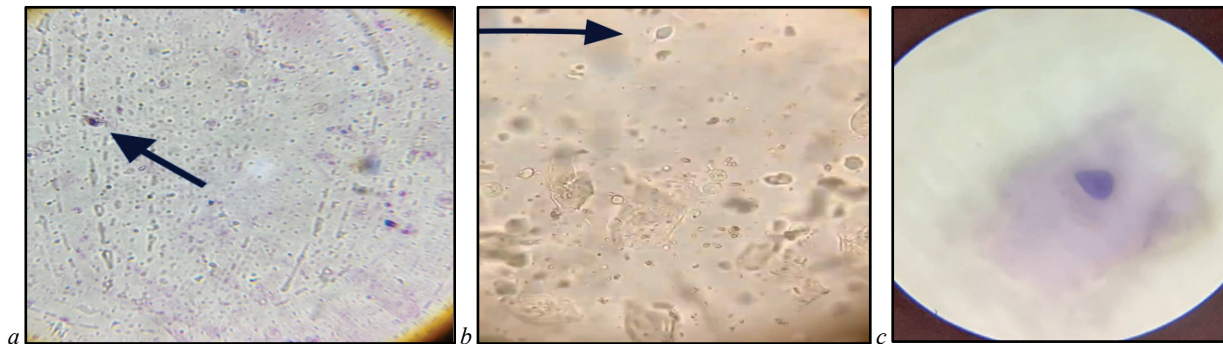


Fig. 1. *Trichomonas vaginalis* (different stages; Giemsa): *a* – refer to trophozoite while *b* refers to direct examination without conventional stain preparation view; *c* – *T. vaginalis* parasite after staining with Giemsa stain

Table 1
Contents of PCR Reaction mixture

Components	Volume, μL
DNA from samples	5.0
F: primer	1.5
R: primer	1.5
Master mix	12.5
H ₂ O for PCR	4.5
Total volume	25.0

A 1.5%-agarose gel electrophoresis was utilized at 80 volts for 60 mins. The products were visualized by a UV-machine.

The results revealed the presence of the parasite in 64 (64%) of woman swabs samples as detected by the PCR (Fig. 2).

Table 2
PCR Program for 18S rRNA gene amplification (repeat – 35 cycles)

Stage	Temperature, $^{\circ}\text{C}$	Time, seconds
Initial denaturation	94	5X60
Denaturation	94	45
Annealing	50	35
Extension	72	60
Final extension	72	5X60

Table 3
Rate of infection of *Trichomonas vaginalis* in women by polymerase chain reaction according to the geographic area ($\chi^2 = 0.298$, $P = 0.585$)

Geographical area	Positive cases	Total number
Urban	18	30
Rural	46	70
Total	64	100

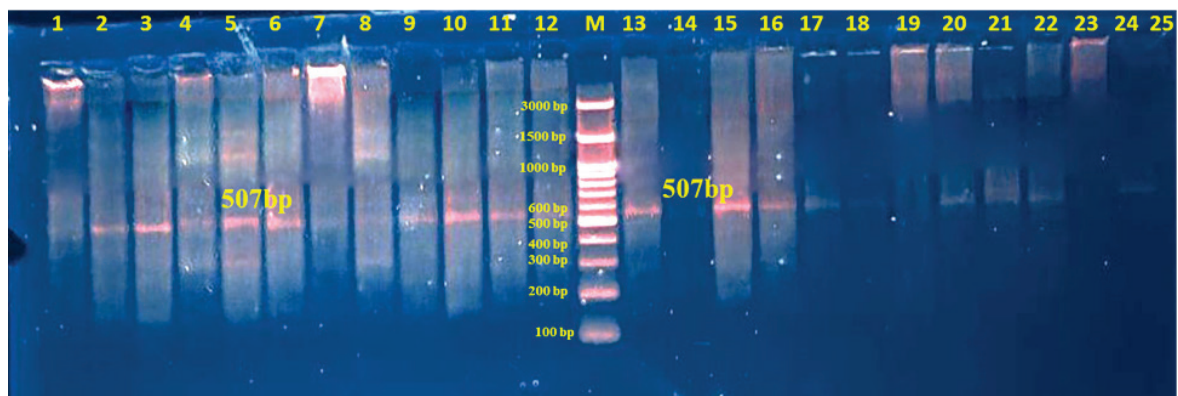


Fig. 2. PCR based on ITS1 of *Trichomonas vaginalis* isolated from vagina of women, products at 507 bp (1–25): Ladder (M):3000 bp; 13 – positive control, 14 – negative control

Table 4
Rate of infection of *Trichomonas vaginalis* in women by polymerase chain reaction according to the age group ($\chi^2 = 16.08$, $P < 0.0001$)

Age interval	Positive	Total number
20–25	20	40
26–35	28	30
36–40	16	30
Total	64	100

Table 5
Primers 18S rRNA used for the detection of *Trichomonas vaginalis*

Primer	Sequence 5'–3'	Amplicon size	References
F	GCGTAAACACGCCCGTATC	507	Sequence: XR_007835997.1
R	GACCGAAGCCTTGTCAGTCA		

Discussion

Trichomonas vaginalis is a very common and serious anaerobic eukaryotic protozoan parasite, which is transmitted through unsafe sexual intercourse (Čepička et al., 2016). *Trichomonas vaginalis* is a parasite that causes trichomoniasis, which is considered as one of most serious diseases transmitted sexually worldwide (Mushref et al., 2011; Mosa et al., 2022). This parasite causes different and complex symptoms. It affects mainly women. The incidence of trichomoniasis among men is very low and in the majority of cases asymptomatic (Hillier et al., 2021). Often there is no sign or symptom of the illness. Furthermore, it is related to an indigenous microorganism that is found within the genital ducts and urinary tract (Abodulhab et al., 2011). The incidence of *T. vaginalis* within villages in our study area was 65% infected females (46 out of 70) and the incidence rate in urban areas was 60% infected females (18 out of 30).

The statistical analysis shows that there are no significant differences between the incidence of the parasites in different areas (Table 3) and the overall rate of 64% is comparable to the results of studies conducted by Saleh et al. in Khartoum, Sudan in which 85% of 297 women were reported to have this parasite in their vaginal secretion. In contrast to this, a study by Falk et al. (2005) in Egypt found no infection in 91.3% of examined women. Similarly, Dawood et al. (2013) reported an infection rate of 2.8% of females from a sample size of 1600 in hospitals in Kirkuk city, Iraq.

Health personnel like to prescribe Flagyl Metrandiazole, an anti-inflammatory drug, which eradicates the parasite and they try to raise awareness of sexual health among mothers attending delivery clinics (Abodulhab et al., 2011).

In studies conducted in Malaysia (Amal et al., 2010) and Nigeria (Omoriegie et al., 2010), *T. vaginalis* was reported in 0.37% of cases of women seeking treatment at clinics. In the Malaysia case, this finding may be due to women of the Chinese community using herbal lotion which is applied to the vagina for prophylaxis and treatment of STDs. The prevalence of trichomoniasis is caused by lack of attention and awareness of the danger to health from sexually transmitted infections. Transmission varies among different communities. Variation may be due to differences in the sample size, in the period of study, variation in methodology of research and method of diagnosis for trichomoniasis and variation in the social norms of sexual behavior and number of partners in different regions of the world. This is evident in differences in rate of incidence (Dawood et al., 2013). In our study, the infection rate of *T. vaginalis* for women in age group (20–25) was 20 out 40 (50%) while in the age group (26–35) it was 28 out 30 (93.3%), and in age group (36–40) 16 out 30 (53.3%). The exceptionally high infection rate in the age group (26–35) concurs with the findings of Al-Zaidy (2004) in Najaf. Al-Zaidy's study showed that the highest percentage ratio was registered in the age group (25–34) (26.6%). Overall, a low level of education has been associated with a high prevalence of infection as proved by different studies. In Sri Lanka this relationship was observed by Fernando et al. (2012). In the United States this relation was proven by Anang (2010). In Vietnam this phenomenon was also demonstrated by Anh (2012). In Iraq this relationship was found by Dawood et al. (2013). In all those studies, the prevalence of *T. vaginalis* infection was proved to be higher in women with low educational status. The high prevalence of infection in women with a low level of education might be due to their lack of interest in their health status, their lack of knowledge about health education and their inability to have regular visits to gynecology treatment centers. The Center of Disease Control (CDC, 2012) concluded that the parasite is known to live in moist areas of the genitals, such as the urethra or vagina. While it is most commonly passed from one person to the next during sex, it can sometimes be spread through genital contact with wet towels, wet toilet seats or wet clothing. Yet our study differs from the CDC (2012) findings (Krieger, 2010). In his study Krieger used thermal baths to determine the life span of *Trichomonas* and concluded that it can survive on the toilet seat for more than 3 hours. According to the current study, the thermal baths environment is not similar to the toilet seat environment. The two conditions differ and significantly affect the life span of the parasite.

Conclusion

The percentage of women diagnosed with trichomoniasis using PCR assay was greater in rural areas in comparison to urban areas. Also, the majority of females infected with trichomoniasis were sexually active women, aged between 20–40 years old. The high prevalence of *T. vaginalis* infection in the study population is due to several factors. These include personal hygiene, social and demographic factors, lifestyle behaviour, and immunity. Our study recommends conducting research on the infection of *T. vaginalis* in women in health care centers in order to contribute to the implementation of programs to control sexually transmitted diseases.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Both authors contributed equally in this research by preparation, methodology, writing, investigation, and editing.

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