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ORIGINAL ARTICLE

Comparison of Different Techniques for the Diagnosis of Trichomonas Vaginalis Infection in Females at Reproductive Age

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ABSTRACT
Trichomoniasis is a sexual transmitted disease that affects human fertility, abortion, low birth weight, in women, caused by the protozoan parasite Trichomonas vaginalis. The aim of the study was to determine the efficacy of different techniques for diagnosing of trichomoniasis in women. A case control study was carried out 230 women their age from (20-60 years) that divided into two groups (155 as patient groups and 75 as control groups without any symptoms of infection) through the period between November 2020 and July 2021 in Basra Maternity Hospital.The detection rate of T. vaginalis infection was 10 (38.5%), 17 (65.4%), 18 (69.2%), 21 (80.8%) and 25 (96.2%) using wet mount, Giemsa stain, AO, culture and in pouch system, respectively. The infection indicates that the highest frequency of positive samples 10 (13.2 %), 18(14.2%) ,21(15.8%) in young women aged 20-30 years, in rural areas and in women with primary education, respectively. The highest rate of infection with T. vaginalis was detected by in pouch system in this study offers a useful rapid screening tool and provides an excellent alternative for definitive laboratory diagnosis of T. vaginalis, thus reducing spread and transmission of the infection as well as minimizing the complication sequels.
Keywords: Trichomonas vaginalis, Acridine orange, Diamond modified culture, in pouch TV.

INTRODUCTION
Trichomoniasis is the most common non-viral sexually transmitted infection in the world, caused by the protozoan parasite Trichomonas vaginalis, which infects the urogenital tract of men and women. Approximately, 250 million new cases of Trichomonas vaginalis Infection are reported worldwide each year [1; 2]. The infection is generally acquired through sexual contact and sometimes through non-sexual contact, including fomites [3]. Trichomoniasis often leads to vaginitis in women and urethritis and prostatitis in men. Moreover, infection with T. vaginalis in pregnant women has been associated with premature rupture of membranes, premature labor, abortion, low birth weight, and increased infant mortality [4]. Another serious aspect of trichomoniasis is its relationship with an increased risk for human immunodeficiency virus (HIV) and human papillomavirus (HPV) infections [5]. It can also cause prostatitis, reduced sperm function, infertility, and higher risk of prostate cancer in men [6; 7]. Diagnosis of this infection is still a critical aspect. Correct diagnosis of trichomoniasis will help to reduce obstetric and gynecological complications. [8; 9]. Several methods of diagnosis of trichomoniasis exist. There is the easiest method which involves examination of a wet preparation under the microscope where the organisms are seen moving rapidly in all directions. Other methods include overnight culture [10]. The aim of the present study was to assess the rate of T. vaginalis infection and the associated risk factors among women attending the Emergency Unite and Department of Obstetrics and Gynecology of Basrah city, Iraq. In addition, specified of direct wet mount and two staining methods (Giemsa and Acridine Orange) compared using Diamond's media culture & In pouch TV as the gold standard. [11].

MATERIALS AND METHODS
The study participants: A case control study was carried out 230 women their age from (20-60 years) that divided into two groups (155 as patient groups and 75 as control groups without any symptoms of infection) through the period between November 2020 and July 2021 in Basra Maternity Hospital, the main Centre for gynecology and obstetrics in the city. The study did not include patients being treated for vaginitis or cervicitis. Patients under treatment of vaginitis or cervicitis were excluded.
Sample collection: Using sterile vaginal swabs, speculums were used to collect three specimens of vaginal discharge from the posterior vaginal fornix. Approximately one ml of normal saline was added on to the first swab and squeezed onto the tube wall for use in a wet mount smear and staining within one hour. In the second swab, Diamond modified medium culture tubes were immersed and squeezed for cultivation. The third swab was carefully inoculated into a pouch TV culture system.
Wet preparation method: According the procedure [12; 13]. (Fig. 1-A-).
Staining:
Giemsa-stained smear: According the procedure [14].

On the microscopic glass slide, one drop of the vaginal swab-saline solution was smeared on, and then fixed the slide in methanol for 30 minutes. Stains were applied in a Giemsa dye solution for 2 to 3 hours (timing was modified based on preliminary trials), followed by rinsing under running water then they were vertically positioned to dry. 1000X microscopy was used to detect violet, pear shape trophozoites on the slides. (Fig. 1-B-).
Acridine Orange Staining: Using a vaginal swab-saline suspension, one drop was applied to a microscopic glass slide; it was air-dried, heat-fixed, then immersed for 20 seconds in the stain. After exposing to pH 7.2 buffers, slides were stored in the dark at room temperature, until examined [15]. A TS 510 nm selective beam splitter was used to scan wet slides under fluorescent microscope at 400 X, there are three filtering components: a G247 nm barrier filter, a G249 nm additional filter, and an excitation filter for narrow-band excitation of 255 nm. trophozoites of T. vaginalis with a brick red color and a yellow-green nuclei (Fig. 1-C-). Bacteria and yeast stain bright red, but they are much smaller and morphologically different from trichomonads, so they are easily distinguished.
In vitro cultivation of T. vaginalis: Anaerobic conditions were used for incubation at 37 °C for 7 days with the swab specimen in Diamond's culture medium [16]. Wet mount smears of culture are examined daily. Diamond's medium (pH 6.0) without agar, containing 10% inactivated horse serum, was inoculated with T. vaginalis, and incubated at 37°C. A milliliter of old cultured in 2-day- T. vaginalis (approximately 0.5–1.0 × 10^6 parasites/ml) in log phase growth. Centrifugation at 16,000 * g for 10 seconds followed by resuspension of the pellet into 50 ml of Diamond's medium containing 50 % newborn lamb serum or resuspension in 50 ml of Diamond's medium containing parasites.
In pouch TV culture system: Vaginal swabs were inoculated into the upper chamber of an In Pouch TV culture system (Biomed Diagnostics, Santa Clara, CA, USA). The upper chamber contents were immediately pushed into the lower chamber, followed by incubation at 37°C. A microscopy examination of the cultures was performed on days 2, 3 and again on day 5 after inoculation. positive result showed the presence of motile trichomonads. [17].

Socio-demographic profile study among women with Trichomonas vaginalis infection: Each patient was provided with informed consent and examined by a gynecologist to obtain vaginal swabs. Full information history was taken directly from the patient such as symptoms, age, education, Residence.

Statistical analysis: Statistical Package for Social Science (SPSS) was used to analyze the data. The results were expressed as numbers, percentages, and mean ± S.D. (standard deviation). ANOVA was used to evaluate differences between groups using $p \leq 0.05$ lowest limit of significance.

RESULT

Rate of T. vaginalis Infection: The study, determine the efficacy of different techniques for diagnosing of trichomoniasis in clinical laboratories.

Out of 230 cases women was examined, 26 of them (11.3%) are harbouring T. vaginalis by at least one of the five techniques used. The largest number of positive findings was 25 (96.2%) recorded with in pouch system in addition the other 1 was diagnosed using the culture technique & the lowest number was found in Wet mount techniques 10 (38.5%) with statistically significant difference was found between them ($p<0.001$) (table 1). There were quite marked differences between the infection rates revealed by these two tests and with culture 21(80.8%), Giemsa-stained smears 17(65.4%) and Acridine Orange was18 (69.2%) Table (1).

Table 1: Comparison of diagnosis vaginal trichomoniasis in different methods

Diagnosis	No. positive %	No. Negative %
Wet preparation	10 (38.5%)	16(61.5%)
Giemsa Stain	17(65.4%)	9(34.6%)
Acridine Orange	18(69.2%).	8(30.8%)
Culture in Modified Diamond medium	21 (80.8%)	5(19.2%)
In pouch TV	25 (96.2%)	1 (3.8%)

Socio-demographic profile among women with Trichomonas vaginalis infection: Table 2 summarizes the numbers of individuals with T. vaginalis infection stratified for age, residence and education. Figures 1 and 2 shows the percentage of individuals with T. vaginalis in different age groups and different residence. The results indicate that the highest frequency of positive samples were detected in subjects 20-30 years of age (13.2 %) and those between 31 and 40 years old (12.1%). The lowest frequency belonged to patients younger than 50 years old (8.3%). while the highest percent of positive cases in rural areas 18(14.2%) and the lowest percent in urban areas 8 (7.8%).

The study revealed that women with primary education have the highest number and percentage 21(15.8%) while low percentage of infection was seen in high educational level 5 (5.2%)

Table 2: Socio-demographic profile in women vs. detection of Trichomonas vaginalis infection

Variable	No. of cases (%) 230	Positive cases (%)	Negative cases (%)	P-value
Age 20-30 31-40 41-50 Over 50	76(33 %) 107(46.5%) 35(15.2%) 12(5.2%)	10(13.2%) 13(12.1%) 2(5.7%) 1(8.3%)	66(86.8%) 94(87.9%) 33(94.3%) 11(91.7%)	p≤0.05
Mean age ±SD	26.13± 6.26	22.73±1.63	24.9±4.12	
Residence Urban	103 (44.8%)	8(7.8%)	95(92.2%)	P ≤0.00
Rural	127 (55.2%)	18(14.2%)	109(85.8%)	
Education	97 (42.2%)	5 (5.2%)	92 (94.8%)	P ≤0.00
High education				
Low education	133 (57.8%)	21(15.8%)	112 (84.2%)	

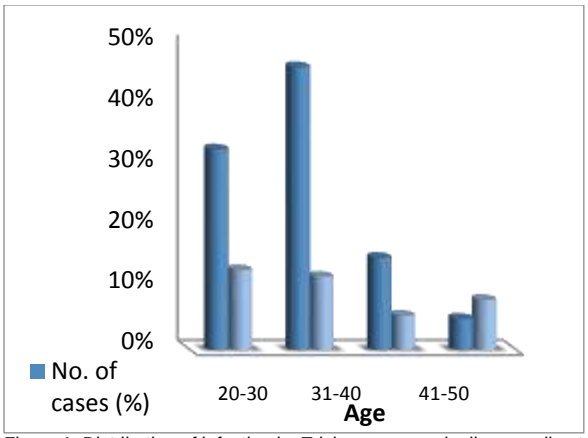


Figure 1: Distribution of infection by Trichomonas vaginalis according to age groups

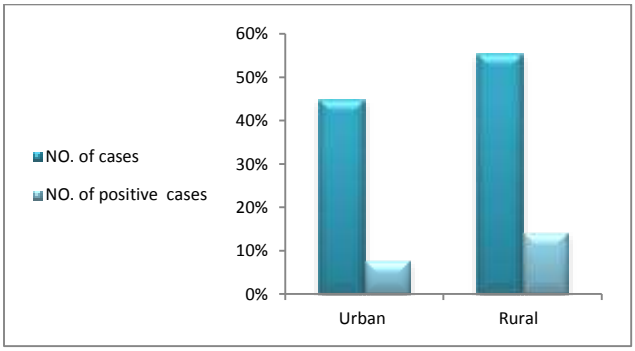
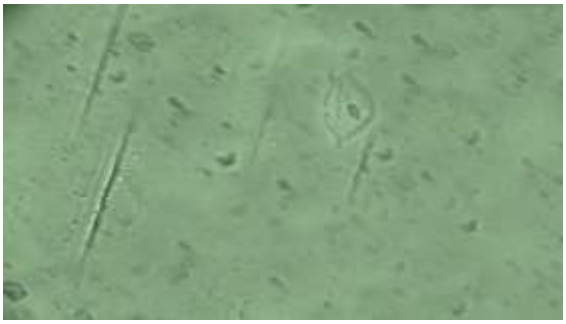


Figure 2: Association between infection by Trichomonas vaginalis and Residence status



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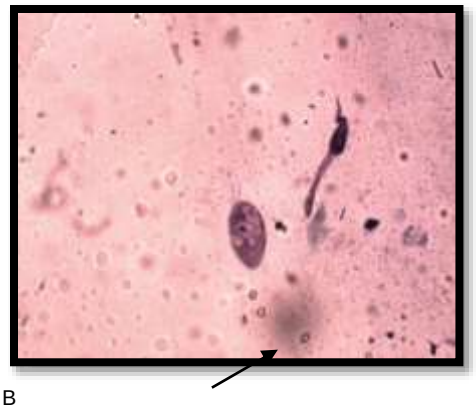

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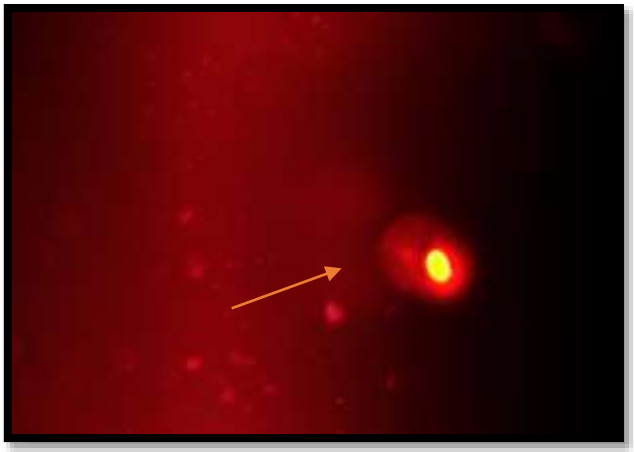

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Figure 3: (A) T. vaginalis under a microscope with a wet amount of 40X without staining. (B) Microscopically viewed under a 40X magnification, T. vaginalis parasite with Giemsa stain. (C) Tropozoite stained Brick Red with Yellowish Nuclei X400 (Acridine orange).

DISCUSSION

Trichomonas vaginitis is one of the most common sexually transmitted infections that affects women between the ages of 16 and 53 (especially pregnant women) and can affect a person's health. In this study, we examined the diagnostic methods of T.V. Studies have shown that wet preparation method, which are common diagnostic methods in the laboratory today; do not have sufficient sensitivity for identification of parasite. Therefore, we should look for alternative methods that can give us the most specific results with less cost and time [18]. Different techniques were used in this study for comparative the rate of infection with T. vaginalis by using wet preparation and Parasite staining by Giemsa stain techniques or Acridine Orange stain, also by cultivation of parasite in Modified Diamond medium or in pouch TV.

The most common method for T. vaginalis diagnosis in women remains microscopic evaluation of vaginal wet preparations due to its low cost and simplicity [19; 20]. In the present study, wet mount is the least specific test with low rate of infection was detect 10 (38.5%) when compared with culture and in pouch TV methods that identified high rate of infection 21 (80.8%) ,25 (96.2%) respectively, it is proved to be highly specific methods. Our results came in accordance with other reports that indicated that wet mount is less sensitive than culture method [21; 22; 23].

The use of staining methods in T. vaginalis infection diagnosis is acceptable. In the present study, the rate of infection was identify by Acridine Orange was 18(69.2%) and by Giemsa Stain 17(65.4%), which are come directly next to that of Diamond's culture. Acridine Orange staining technique is relatively simple to carry out and shows reasonable sensitivity and specificity [24; 25 ;26]. but it requires the use of a fluorescent microscope which is not readily available in all settings.

Our result comes in compliance with other authors who report and confirm that culture remains the most reliable method in the diagnosis of T. vaginalis infection [23]. One limitation of culture method is that it does not allow same day treatment, thus prolongation of the infection, leading to further transmission. [27].

The rate of vaginal trichomoniasis among women enrolled in this study was 11.3% (26/230) this corroborates with previous finding by other researchers who reported the rate of infection in Basrah was 13%, [28]. Higher rate of infection was recorded in Baghdad and Diyala (85.5%), (41.6%) respectively [29; 30]. Globally, prevalence estimates among suspected patients vary between 0.9%-80% [31; 32]. The disparity between different studies could be attributed to many factors including variation in selection of the enrolled study population, the sensitivity of the used diagnostic technique or the skill of the investigator.

In our study, the rate of trichomoniasis has a strong association with age, residence, and education level, which are agree with many studies reported association between risk of T. vaginalis infection with age [33]. race [34] and education [35]. Whereas disagrees with the previous findings [36; 37 ;38].

According to our findings, the highest rate of infection with Trichomonas vaginalis was seen in individuals within 20-30 years of age. The majority of epidemiological studies in Iraq and other countries have evaluated 15–45-year-old, who were sexually active and had referred to gynecology centers [39; 40; 41 ;42].

The present study shows higher rate of infection among women from rural area (14.2%) when compared to women from urban area (7.8%) and these significant differences reported suggest varying level of awareness between rural and urban communities with low, socio-economic status and poor hygiene. [43].

CONCLUSIONS

The highest rate of infection with T. vaginalis was detected by in pouch system in this study offers a useful rapid screening tool and provides an excellent alternative for definitive laboratory diagnosis of T. vaginalis, thus reducing spread and transmission of the infection as well as minimizing the complication sequels.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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