Molecular identification of *Trichomonas vaginalis* compared to culture among women in child-bearing age in West Bank-Palestine

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CHAPTER ONE
1. INTRODUCTION

*Trichomonas vaginalis*, a flagellated parasitic protozoan typically pyriform in culture and amoeboid when adhering to mammalian cells (1); it is the etiologic agent of trichomoniasis, a sexually transmitted disease (STD) of worldwide importance. Trichomoniasis is the most common nonviral STDs, usually surviving only in human urogenital tract and it is associated with many perinatal complications and an increased incidence of HIV transmission (2).

Recent data have shown that the annual incidence of trichomoniasis is more than 180 million cases worldwide (3,4,5), World Health Organization (WHO) estimates that *T.vaginalis* accounts for approximately half of all curable sexually transmitted diseases worldwide (2).

This disease has important medical, social, and economical implications (6). Women who are infected during pregnancy are predisposed to premature rupture of the placental membranes, premature labor, and low-birth-weight infants. Also linked to this disease are cervical cancer, atypical pelvic inflammatory disease, and infertility (2, 7, 8).
*T. vaginalis* was first described by Donné in 1836. Until the 1950s, this organism was recognized as commensal (9). Subsequently, its role in causing the sexually transmitted disease (trichomoniasis) was recognized. Research on this organism has been in a progression of phases throughout the last 60 years (2). During the 1960s and 1970s, research had focused on biochemical tests and microscopic examination to understand the growth characteristics and behavior of the organism. It was not until the 1980s that immunologic methods and molecular biological techniques became available and were applied to study the pathogenesis and immunology of this organism (2).

### 1.1 Morphology and life cycle

*Trichomonas vaginalis* is a microscopic, motile, anaerobic and flagellated protozoan parasite found in women’s vagina and men’s urethra. *T. vaginalis* contains five flagella, four of which are located at its anterior portion; the fifth flagellum is incorporated within the undulating membrane.

*T. vaginalis* possesses no mitochondria, instead producing some of its ATP in hydrogen-producing organelles called hydrogenosomes. It has a nucleus, an axostyle, a glycogen-rich cytoplasm, a variety of
vacuoles (including lysosomes), and numerous micro-tubules with varied structures (2,10,11). The axostyle is a distinctive feature of \textit{T. vaginalis} that runs the length of the organism and appears to protrude from the posterior end. It is a cytoskeletal element composed of concentric rows of microtubules and is believed to function in the attachment of the parasite to epithelial cells.

The life cycle of \textit{T. vaginalis} is simple in that the trophozoite is transmitted through coitus and no cyst form is known. The trophozoite divides by binary fission and, in natural infections, gives rise to a population in the lumen and on the mucosal surfaces of the urogenital tracts of humans (2, 10). The incubation period typically ranges from 3-28 days (12).

\textbf{1.2 Clinical manifestation}

Trichomoniasis can be symptomatic or asymptomatic in both sexes. Men are most frequently considered to be asymptomatic carriers of \textit{T.vaginalis}, representing an important vector and reservoir (13). Demonstration of this organism in men has always been difficult.
Trichomoniasis has been associated with vaginitis, cervicitis, urethritis, and adverse birth outcomes (6, 14, 12). Infection with *T. vaginalis* could have an important role in transmission and acquisition of HIV (9, 12, 15). Other complications associated with trichomoniasis include increased risks for the following: infertility, development of atypical pelvic inflammatory disease, infection following gynecologic surgery, and cervical inflammatory neoplasia. There have also been high rates of correlation between trichomoniasis and pregnancy complications in women. In men, *T. vaginalis* has been linked to male factor infertility and as a common cause of non-gonococcal urethritis (NGU) (7, 8, 16).

The primary symptom of trichomoniasis in women is a copious, foul-smelling vaginal discharge that is often accompanied by burning or itching but approximately half of all women will be asymptomatic (6), and thus screening for disease is important.

This discharge is most often gray, but can be yellow or green and is occasionally frothy or blood tinged. Many women may also experience painful or difficult coitus. Urethral involvement occurs in a large number of cases and is characterized by dysuria (painful
urination) and frequent urination. The absence of discharge therefore does not rule out the infection, nor should the presence of discharge be taken as the sole criteria for diagnosing *T. vaginalis* (2). Various sociodemographic factors have been correlated with presence of *T. vaginalis*, and may be used to predict infection. It is rarely reported in females before puberty and after menopause, but is common during the child-bearing years and peaks during pregnancy (6).

### 1.3 Pathophysiology

The normal physiologic vaginal discharge comprises vaginal secretions, exfoliated cells and cervical mucus. The frequency of vaginal discharge varies with age, menstrual cycle, pregnancy and use of oral contraceptives. The normal vaginal environment is characterized by a dynamic interrelationship between *Lactobacillus acidophilus* and other endogenous flora, estrogen, glycogen, vaginal pH and metabolic by-products of flora and pathogens. *L. acidophilus* produces hydrogen peroxide, which is toxic to pathogens and keeps the healthy vaginal pH between 3.8 and 4.2. Vaginitis occurs because the vaginal flora has been altered by the introduction of *Trichomonas vaginalis* or changes in the vaginal environment that allow pathogens to proliferate (17).
Antibiotics, contraceptives, sexual intercourse, douching, stress and hormones, deodorants soaps, vaginal sprays, tampons or pads, colored or perfumed toilet tissue, bubble bath, laundry detergents (especially those with enzymes), fabric softeners, frequent minipad use, tight-fitting synthetic underwear, swimming pools or hot tubs, and vaginal contraceptives or condoms (3) can change the vaginal environment and allow pathogens to grow (3,17).

In trichomoniasis, it is believed that some inciting event decreases the number of hydrogen peroxide producing *L. acidophilus* organisms (17). The resultant change in pH allows proliferation of organisms that are normally suppressed. These organisms produce metabolic byproducts, such as amines, that further increase the vaginal pH and cause exfoliation of vaginal epithelial cells. In patients with trichomoniasis, changes in estrogen and progesterone levels, as well as elevations of vaginal pH and glycogen levels, may enhance the growth and virulence of *T. vaginalis* (18).

The vagina infected with *T. vaginalis* becomes less acidic and frequently reaches a pH level greater than 5. This favors the overgrowth of bacteria, resulting in increased colonization of the
lower genital tract by anaerobes. This may increase the risk of postoperative infection in the patient who is preparing to undergo pelvic surgery (17).

1.4 Pathogenesis and virulence

Pathogenesis and virulence of trichomoniasis is not fully understood. Adhesion of *T. vaginalis* to vaginal epithelial is believed to be the primary factor contributing to the pathogenesis of the organism. Although several adhesion molecules have been identified on the surface of this parasite, little is known about host cell receptors. There is evidence suggesting that *T. vaginalis* actually binds to the extracellular matrix glycoproteins, laminin and fibronectin (19). It was suggested by many reports that the association of *T. vaginalis* with these two plasma proteins contributes to host parasitism (20).

Application of antibodies to block binding of adhesion molecules to targets on vaginal epithelial cells reduced drastically the adhesion of the parasite to the cells. In addition there was no sign of any cytopathic effects on these cells in vitro (21). Contact of *T. vaginalis* to vaginal epithelium caused morphological changes in the parasite
where it became flattened with concomitant up-regulation in the expression of many genes including those encoding for the adhesion molecules (22).

Cysteine proteinases have been known to play an important role in the virulence of all parasites in general and *T. vaginalis* in particular. Many functions have been attributed to these molecules including adherence and cytotoxicity.

Incubation of live *T. vaginalis* with human vaginal epithelial cell monolayers produced disruption of host cells within two hours and resulted in total loss of cell viability after extended exposure to the parasite (23). Expression of proteolytic activity and localization of adhesion molecules on the surface of the parasite is controlled by the presence of iron. Likewise, the transcription of Ap65-1, an adhesion protein, is regulated by the presence of iron-responsive DNA elements (24).

The cytopathic effect of *T. vaginalis* on host cells is considered to be significant in the pathogenesis of this parasite. *T. vaginalis* is considered as an extracellular parasite that induces cytopathic effect on host cells via two major mechanisms, one is contact-dependent and the other is contact independent. Contact dependent cytopathic
effect is mediated by several cysteine proteinases and hemolytic factors as mentioned previously. It was found that *T. vaginalis* elaborates soluble factors or toxins that have the ability to damage target cells in vitro (25). One toxin or soluble factor was referred to as the lytic factor (LF). LF was isolated, purified and its molecular characteristics were determined. Additional testing on the activity of the lytic factor revealed that it is a phospholipase. LF has the ability to hydrolyze phosphotidylcholine yielding products similar to those produced by phospholipase A2 (PLA2). This indicates that the major enzymatic component of LF that mediates host cell lysis is PLA2. LF is considered to be an important virulence factor of *T. vaginalis* which mediates host cell lysis, tissue damage and inflammation in trichomoniasis (25).

### 1.5 Epidemiology

It is estimated that one in five sexually active women will be infected with *T. vaginalis* in their lifetime. Transmission is mainly by physical contact as *T. vaginalis* can only survive outside the host for a short period of time (26).
In the United States annually, an estimated 8 million women are infected with *T. vaginalis*, and the annual number worldwide is approximately 180 million (3, 4, 5).

Trichomonal infection has been encountered in every continent and climate and with no seasonal variability. It has a cosmopolitan distribution and has been identified in all racial groups and socioeconomic strata. Although trichomoniasis is the most common of STIs, data on prevalence and incidence are limited (27).

In pregnancy, the reported prevalence rate of trichomoniasis ranges from 9% to 47% (28). Several clinical studies reported high incidence rates of trichomoniasis among pregnant women. The prevalence of trichomoniasis amongst pregnant women in Africa show rates ranging from 9.9% in Central African Republic to 41.4% in South Africa (27).

Studies on the prevalence of trichomoniasis in the Mediterranean region have been obtained on women attending family planning, reproductive and gynecological clinics. In Izmir (Turkey), the prevalence of trichomoniasis was found to be 15.4% (29). The prevalence of trichomoniasis among Iraqi women was found to be
11.3%; another study conducted in Basra (Iraq) revealed a prevalence rate of 13% (30). Prevalence was found to be (8.7%) among married women in Upper Egypt (31), while the prevalence in Israel was found to be 8.1% (32).

Humans are the only natural host for *T. vaginalis*. The trophozoite is transmitted from one person to another, usually by sexual intercourse which includes vaginal, oral or anal sex and from a mother to child during birth (2,10). Rarely, *T. vaginalis* is transmitted by sharing towels, flannels, hot baths or jacuzzis. Newborn infants of mothers infected by *T. vaginalis* have on occasion acquired a *T. vaginalis* urinary tract or vaginal infection. The organisms were acquired by 2 to 17% of female neonates of infected women (33). In addition, neonates have been infected during the birth process. Newborn girls can acquire the infection from their infected mothers during passage through the birth canal. In such cases, the infection tends to remain asymptomatic until puberty (11, 34, 35).

Finally, trichomoniasis facilitates the spread of HIV epidemic. Theoretically, calculations concluded that infection with *T. vaginalis* increased the risk of HIV transmission by 90%. In a population with
25% prevalence of trichomoniasis, approximately 20% of cases of HIV would be attributable to trichomoniasis. Rates of trichomoniasis and HIV seem to be directly proportional (36).

1.6 Treatment

The standard treatment for trichomoniasis is 250 mg of metronidazole, given orally, three times a day for 7 days, or in a single 2g-dose. Both the infected patient and sexual partner, whether symptomatic or asymptomatic, should be treated to prevent reinfection (27,37). The single 2-g dose of metronidazole is the treatment currently recommended by the Centers for Disease Control and Prevention for women at any stage of pregnancy, also for lactating mothers followed by a 24-h interruption in breast feeding to prevent neonatal exposure to drug (37).

As regarding prevention and treatment, both partners should receive careful examination and treatment. Furthermore, medical examination instruments must be sterile in order to prevent transmission of the disease. Routinely screening and treating women for *Trichomonas vaginalis* before any reproductive tract surgery and also before pregnancy and after delivery or abortion may help to
prevent the occurrence of infection. Barrier protection (condoms) should be used during intercourse until the infection is eradicated in both partners. The population must be educated about this disease and the means of transmission, since education on sexual behaviour and genital hygiene may help in its prevention and control (37).

1.7 Diagnosis

In areas where diagnostic methods are limited, management of trichomoniasis is usually as part of a clinical syndrome for women characteristics of the vaginal discharge, including color and odor, which are poor predictors of *T. vaginalis* (29, 31). Since no symptom alone or in combination is sufficient to diagnose *T. vaginalis* infection reliably, laboratory diagnosis is necessary (36).

Traditionally, diagnosis of trichomoniasis in women has relied on microscopic examination of wet mount preparations made directly from vaginal secretions. Although rapid and inexpensive, this technique is relatively insensitive, has a low sensitivity of 36-75% (5,38, 39, 40). When motility is lost, it is difficult to distinguish *T. vaginalis* from white blood cells and there is usually an increased number of polymorphonuclear white blood cells with *T. vaginalis*
infection (41). Studies have reported a range of false negatives on wet mounts from 23% to 58% (41). Therefore, it is necessary to examine fresh specimens as quickly as possible after collection. Culture of trichomonads from vaginal swab specimens and urine (16) remains the "gold standard" against which the performance of other diagnostic methods is measured. Unfortunately, culture is time-consuming, with results usually being available only 48 to 72 h after inoculation of the culture medium. Also, it is impractical in many settings since specialized laboratory equipment such as incubators and microscopes is required.

Recently, a number of PCR assays (42,43) have been developed for the detection of *T. vaginalis* and these assays have generally proved more sensitive than culture; however, PCR also requires a dedicated laboratory, sophisticated equipment, and specially trained laboratorians.

Guillermo et al. (44) developed a PCR method using primers that produced a segment of B-tubulin sequence with 97% sensitivity and 98% specificity. Their primers did not amplify genes of other species of trichomonas, intestinal flagellates and bacterial agents of vaginitis. Patel found that the sensitivity of the polymerase chain reaction
technique was 95% and the specificity was 98% compared to other diagnostic tests (45).

Based on different studies, PCR is very sensitive and specific for detection of *Trichomonas vaginalis* suggesting this method as the gold standard instead of other screening methods (44, 46).

### 1.8 Statement of the problem

Trichomoniasis is the most common sexually transmitted diseases (STDs) worldwide. It is caused by the parasite *Trichomonas vaginalis* (TV). An estimated 180 million new infections occur annually (3,4,5). In the United States the incidence was found to be 8 million new cases annually (5).

According to the Palestinian Ministry of Health, reporting of sexually transmitted disease is categorized according to etiological causes and syndromic diagnosis. The number of cases for patients with trichomoniasis in Palestine was estimated to be 103 cases in females and 36 cases in males per 100,000 (47).

Clinical studies have demonstrated that trichomoniasis may have serious side effects both in pregnancy and in a predisposition to
retrovirus infection (27). T. vaginalis causes a common genitourinary infection, which is frequently asymptomatic; it has been estimated that 10-50% of T. vaginalis infections in women are asymptomatic (11, 19, 47, 48). Infestation with TV is a common and potentially morbid infection. In addition to reproductive tract discharge and irritation, infection with these protozoa is increasingly to be associated with reproductive tract complications, including post abortion infection, post cesarean infection, preterm birth (38,39) and low birth weight infants. T. vaginalis infection has been suggested also as a risk factor for developing cervical cancer. Other complications of T. vaginalis include pelvic inflammatory disease, tubal infertility, vaginitis and urethritis (19,38,39).

T. vaginalis is a common pathogen that is associated with diverse pregnancy outcomes and may serve as a cofactor in human immunodeficiency virus (HIV) transmission (36,38,39).

In this study, a PCR targeting the beta-tubulin genes of T. vaginalis was used for the detection of the organism in vaginal swab samples. The targeted genes encode the amino acid sequence of
beta-tubulin protein, a major component of the *T. vaginalis* cytoskeleton.

1.9 Specific Aims:

The specific aims of this study were:

1. To determine the infection rate of *T. vaginalis* among women in childbearing age in the West Bank - Palestine.

2. To establish baseline data on the infection rate of trichomoniasis that could help the policy makers in Ministry of Health to take decisions for control and prevention of the infection according to data obtained.

3. Determine the distribution of trichomoniasis in the West Bank.

4. Determine the age distribution of the disease according to age.

5. To evaluate current culture methods using Trichomonas medium No. 2, for the purpose of improving detection methods for *T. vaginalis*.

6. To adopt molecular amplification methods (PCR) using specific primers (BTUB 9/2) to identify *T. vaginalis* infections.
CHAPTER TWO
2. MATERIALS AND METHODS

2.1 Vaginal swabs:

A total of 1207 women in childbearing age attending the governmental, UNRWA and private sector reproductive health clinics in the West Bank-Palestine, between October 2004 and June 2006 were screened for trichomoniasis which is considered one of the most commonly sexually transmitted diseases. Samples were collected by physician of the clinic after getting the women's written consent on a special form prepared for the purpose of this research; vaginal pH level was determined by placing litmus paper against the lateral vaginal wall.

The number of samples was selected according to the estimated population in the year 2003 (see annex 1). Samples were collected randomly from women in all districts in the West Bank-Palestine. Vaginal swabs were collected and sent immediately to the Central Public Health Laboratory in Ramalla. Within 6-8 hours of sample collection, samples were cultured in the broth medium Trichomonas medium No. 2, then incubated at 37°C for up to 5 days. Microscopic wet mount examination of the broth was performed daily
for five days. All positive cultures were stored at – 70°C for further molecular analysis.

The questionnaire was designed according to WHO recommendations for sexually transmitted infections and HIV (see annex 2, 3).

**2.2 Examination of *Trichomonas vaginalis* by Culture:**

Swabs sent to the laboratory from the collection clinics were immediately used to inoculate the medium by cutting the swabs and putting it in the culture medium, and then cultures were incubated at 37°C for 24-hrs to check up the motility of *Trichomonas vaginalis* microscopically. Positive cultures stored at -70°C for PCR. Were if the sample was negative, it was reincubated and checked daily for 5 days.

Trichomonas medium No.2 (Oxoid) was selected for the isolation of *Trichomonas vaginalis*. This medium contains liver digest, iron, glucose, chloramphenicol, tryptone soy broth CM129, horse serum SR35 for enrichment, calcium pantothenate (water soluble) and distilled water.
2.3 DNA Extraction:

DNA samples were prepared by a rapid boiling method (8, 13). A 100 µl of *T. vaginalis* cultures were mixed with 400 µl of sterile water, 15µl of lysis solution (64 % Guanidine thiocyanate, Tris-HCL buffer, 3% Dithiothreitol, <1% glycogen) and were boiled at 100°C for 10 min. Then, these preparations were then centrifuged at 14,000 × g for 15 min.

2.4 PCR:

PCR has been used for amplification of a fragment of the beta-tubulin gene of *T. vaginalis*. This was developed for the detection of the organism in vaginal swab samples utilizing the BTUB 9/2 primer set. The beta-tubulin genes encode beta-tubulin protein, a major component of the *T. vaginalis* cytoskeleton.

2.4.1 PCR primers:

The primers (BTUB 9/2) used in this study were designed to target a conserved region of the beta-tubulin genes of *T. vaginalis*. To improve the specificity, primer sequences were selected from the regions of the *T. vaginalis* beta-tubulin genes that differed
substantially from the beta-tubulin gene sequences of humans and other microorganisms. The primer set used in this study was:

BTUB 9, 5’ CAT TGA TAA CGA AGC TCT TTA CGA T 3’ (9/25)

BTUB 2, 5’ GCA TGT TGT GCC GGA CAT AAC CAT 3’ (2/24)

2.4.2 PCR procedure

PCR was performed in a total volume of 50-100 µl for each specimen. The PCR mixture contained: 0.25 µM of each primer, 0.1 mM deoxynucleoside triphosphates, 1X PCR buffer (10 mM Tris HCl [pH 8.4], 50 mM KCl), and 0.5 mM MgCl2, 0.5 U of Taq polymerase (PeqLab-Germany); 10 µL template DNA were added to respective tubes.

DNA amplification was performed in a thermocycler (BioMed, Theres, Germany Theres). The PCR consisted of 40 cycles each consisting of denaturation at 94°C for 30s, annealing at 63°C for 30s, and extension at 72°C for 1 min. A precycling denaturation at 94°C for 5 min and a final extension at 72°C for 7 min were also applied for each PCR run. Ten microliters (10µl) of PCR products
were electrophoresis on a 2 % agarose gel containing ethidium bromide (0.5 mg/ml).

Positive and negative controls were included in all PCR runs. DNA extract from a clinical isolate *Trichomonas vaginalis* grown in Trichomonas medium No.2 was used as a positive control.

**2.5 Gel Electrophoresis**

The electrophoresis was performed in 1× TBE at a constant current of 80 V for 2-hrs. The gels were viewed and photographed under UV transillumination. The sizes of the amplified products were assessed by comparison with a commercial 100-bp molecular weight ladder (PeqLab-Germany).
CHAPTER THREE
3. Results

A total of 1207 women attending the reproductive health clinics were screened for *T. vaginalis* infection. A positive result was defined as the presence of motile trichomonads upon the microscopic examination of a wet mount preparation from culture enrichment of *T. vaginalis* from vaginal swabs.

Of the 1207 women specimens cultured on broth containing trichomonas medium No. 2, 164 cultures (13.6 %) were found to be positive for *T. vaginalis* (Table 1). Several variables were taken into consideration during this study. These variables including residency distribution (Table 2), age (Table 3), marital status, pregnancy and lactation (Table 4), women using contraceptives (Table 5), socioeconomic conditions, education (Table 6) and manifestation of clinical symptoms (Table 7).

For a total of 1207 women examined in the West Bank, 164 (13.6 %) women were found to be infected with *T. vaginalis*. The high infection rate was observed in Hebron and Salfit.
Table 1: Distribution of infection rate of *T. vaginalis* by districts.

<table>
<thead>
<tr>
<th>West Bank</th>
<th>No. examined</th>
<th>No. positive (culture)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jerusalem</td>
<td>60</td>
<td>10</td>
</tr>
<tr>
<td>Jericho</td>
<td>55</td>
<td>7</td>
</tr>
<tr>
<td>Bethlehem</td>
<td>110</td>
<td>11</td>
</tr>
<tr>
<td>Hebron</td>
<td>301</td>
<td>57</td>
</tr>
<tr>
<td>Tulkarem</td>
<td>101</td>
<td>11</td>
</tr>
<tr>
<td>Nablus</td>
<td>185</td>
<td>29</td>
</tr>
<tr>
<td>Qalqilia</td>
<td>53</td>
<td>7</td>
</tr>
<tr>
<td>Jenin</td>
<td>174</td>
<td>16</td>
</tr>
<tr>
<td>Salfit</td>
<td>35</td>
<td>7</td>
</tr>
<tr>
<td>Ramallah</td>
<td>133</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>1207</td>
<td>164</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>District</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
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<td>164</td>
</tr>
</tbody>
</table>

There was no significant difference of infection by residential area despite that infection was more common among women living in refugee camps (18.6%) as shown in Table (2).

Table 2: Infection rate of *T. vaginalis* according to residency distribution.

<table>
<thead>
<tr>
<th>District</th>
<th>No. examined</th>
<th>No. positive (culture)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban</td>
<td>483</td>
<td>69</td>
</tr>
<tr>
<td>Rural</td>
<td>665</td>
<td>84</td>
</tr>
<tr>
<td>Camps</td>
<td>59</td>
<td>11</td>
</tr>
</tbody>
</table>

| Total    | 1207         | 164                    |

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<tr>
<td>Rural</td>
<td>665</td>
<td>84</td>
</tr>
<tr>
<td>Camps</td>
<td>59</td>
<td>11</td>
</tr>
</tbody>
</table>

| Total    | 1207         | 164                    |

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<tr>
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<th>No. examined</th>
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</tr>
</tbody>
</table>

| Total    | 1207         | 164                    |

There was no significant difference of infection by residential area despite that infection was more common among women living in refugee camps (18.6%) as shown in Table (2).
The highest infection rate (20.7 %) of *T. vaginalis* was observed among women 18 years old and younger (Table 3). In contrast the lowest infection rate (6.3 %) was observed among women whose age were 46 years and older as shown in Table 3.

Married women showed an infection rate of 13.6 % and widows showed an infection rate of (14.3%). However, the total number of widows who participated in this study was only 7, which did not allow a reliable statistical evaluation of the infection rate in this group. Additionally, there were 3 divorced women in the study all of which were negative for trichomoniasis.

Table 3: Infection rate of *T. vaginalis* according to age

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Number/group</th>
<th>Positive cases (culture) No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-18</td>
<td>29</td>
<td>6</td>
<td>20.7</td>
</tr>
<tr>
<td>19-25</td>
<td>268</td>
<td>42</td>
<td>15.7</td>
</tr>
<tr>
<td>26-30</td>
<td>262</td>
<td>36</td>
<td>13.7</td>
</tr>
<tr>
<td>31-35</td>
<td>264</td>
<td>32</td>
<td>12</td>
</tr>
<tr>
<td>36-45</td>
<td>314</td>
<td>44</td>
<td>14</td>
</tr>
<tr>
<td>Over 46</td>
<td>64</td>
<td>4</td>
<td>6.3</td>
</tr>
<tr>
<td>Total</td>
<td>1207</td>
<td>164</td>
<td>13.6</td>
</tr>
</tbody>
</table>
Considering the infection rate in pregnant and lactating women, it was found that pregnant women showed a higher and statistically significant infection rate (28.1 %) as compared to lactating women (11.4 %) as well as women not belonging to these two groups (10.9 %) - neither pregnant nor lactating- (Table 4). However, no statistically significant difference in the infection rate was observed between lactating women and other women not belonging to pregnant or lactating groups (Table 4).

Table 4: Infection rate of *T. vaginalis* according to status of women.

<table>
<thead>
<tr>
<th>Status</th>
<th>n</th>
<th>Positive cases (culture)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Pregnant</td>
<td>178</td>
<td>50</td>
</tr>
<tr>
<td>lactating</td>
<td>324</td>
<td>37</td>
</tr>
<tr>
<td>others</td>
<td>705</td>
<td>77</td>
</tr>
<tr>
<td>Total</td>
<td>1207</td>
<td>164</td>
</tr>
</tbody>
</table>

* Others: women who were neither pregnant nor lactating

*T. vaginalis* infection was more common among women who did not use contraceptives (17.1 %) than among those using oral contraceptive (9.2 %), IUD (11.6 %), (Table 5), condoms (11.1 %)
and to a lesser extent among women using injections (14.3 %) (Table 5).

Table 5: Infection rate of *T. vaginalis* according to contraceptive methods.

<table>
<thead>
<tr>
<th>Contraceptives</th>
<th>n</th>
<th>Positive cases (culture)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>IUD</td>
<td>458</td>
<td>53</td>
</tr>
<tr>
<td>Pills</td>
<td>130</td>
<td>12</td>
</tr>
<tr>
<td>injection</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Condom</td>
<td>81</td>
<td>7</td>
</tr>
<tr>
<td>Not-Use</td>
<td>594</td>
<td>91</td>
</tr>
<tr>
<td>Total</td>
<td>1207</td>
<td>164</td>
</tr>
</tbody>
</table>

* %: number of positive cases per group / number of women per group

On the other hand, the infection rate of *T. vaginalis* among housewives (13.7%) was higher than working women (11.7%), and among women with elementary education (13.8%) was higher than among women with higher education (11.4%) (Table 6). Additionally, a comparison of the infection rate among various socioeconomic classes, low, moderate and high income showed an infection rate of 14.4 %, 12.8 %, 15.8% respectively. However there
was no statistical difference observed in the infection rate among the different women’s groups (mentioned previously and shown in Table 6).

Table 6: Infection rate of *T. vaginalis* according to occupation, education and economic status.

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Occupation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Working outside</td>
<td>77</td>
<td>9</td>
<td>11.7</td>
</tr>
<tr>
<td>Housewife</td>
<td>1130</td>
<td>155</td>
<td>13.7</td>
</tr>
<tr>
<td><strong>Educational level</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>1084</td>
<td>150</td>
<td>13.8</td>
</tr>
<tr>
<td>High</td>
<td>123</td>
<td>14</td>
<td>11.4</td>
</tr>
<tr>
<td><strong>Economic status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>542</td>
<td>78</td>
<td>14.4</td>
</tr>
<tr>
<td>Moderate</td>
<td>646</td>
<td>83</td>
<td>12.8</td>
</tr>
<tr>
<td>High</td>
<td>19</td>
<td>3</td>
<td>15.8</td>
</tr>
</tbody>
</table>

Furthermore, Table 7 shows the relationship between the clinical symptoms manifested by the affected women seeking medical help due to vaginitis and the number of cases attributed to *T. vaginalis* as confirmed by the culture method.
Table 7: Infection rate of *T. vaginalis* according to clinical features

<table>
<thead>
<tr>
<th>Presenting symptoms</th>
<th>n</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaginal discharge</td>
<td>990</td>
<td>130</td>
<td>13.1</td>
</tr>
<tr>
<td>Soreness</td>
<td>382</td>
<td>57</td>
<td>14.9</td>
</tr>
<tr>
<td>Urinary symptoms</td>
<td>489</td>
<td>67</td>
<td>13.7</td>
</tr>
<tr>
<td>Pelvic pain</td>
<td>302</td>
<td>48</td>
<td>15.9</td>
</tr>
<tr>
<td>irritation</td>
<td>458</td>
<td>68</td>
<td>14.8</td>
</tr>
<tr>
<td>pH &gt; 4</td>
<td>1125</td>
<td>154</td>
<td>13.7</td>
</tr>
</tbody>
</table>

The percentage of cases presented with increased vaginal pH and vaginal discharge was 79.2 % and 93.9 %, respectively, relative to culture-confirmed infection and 13.7% and 13.1% relative to total study sample respectively.

In this study, 100 specimens were randomly selected from a total of 164 specimens that were positive by the culture method and analysed for presence of *T. vaginalis* by PCR. The DNA was extracted and purified from these 100 specimens and subjected for PCR amplification followed by analysis of PCR amplicons by agarose gel electrophoresis. The primer set BTUB 9/2 used for PCR amplification amplified the predicted 112-bp product in 82 of the selected 100
specimens. A representative agarose gel electrophoresis for PCR products using primer pair BTUB 9/2 shows the successful amplification of the 112-bp fragment from the BTUB gene of *T.vaginalis* (Fig 1). The sensitivity of PCR to identify *T. vaginalis* as compared to culture was 82%.

Figure 1. A representative agarose gel electrophoresis for PCR products

Lane 2:100 bp DNA Ladder, lanes 3-13: culture sample positive for *T.vaginalis*, lane 14: negative control, lane 15: ladder.
CHAPTER FOUR
4. DISCUSSION

Trichomoniasis is the most common nonviral sexually transmitted disease (STD) worldwide. *T. vaginalis* is the third most common cause of vaginitis. World Health Organization (WHO) estimates that *T. vaginalis* accounts for approximately half of all curable sexually transmitted diseases worldwide (6). Annually it affects 180 million women worldwide (3, 4).

The aim of this study was to evaluate and compare new techniques used for accurate identification of *T. vaginalis*. Traditional methods for the identification of *T. vaginalis* rely on microscopic examination (wet mount). Although rapid and inexpensive, this technique has a low sensitivity of 36-75%. In addition, high numbers of the parasite with the characteristic jerky motility must be present for accurate diagnosis to be made. This requires experienced and trained laboratory technician (5, 38, 39, 40).

Culture and amplification techniques (PCR), which are compared in this study, are becoming widely used with high sensitivity and specificity. Traditionally, diagnosis of trichomoniasis is based on clinical grounds. In women, the characteristic vaginal discharge,
including color and odor, are poor predictors of *T. vaginalis* (29, 31). Diagnosis based on symptoms is difficult, since symptoms are not sufficient to diagnose *T. vaginalis* infection reliably. In addition the symptoms of trichomoniasis usually mimic those of other STDs making laboratory diagnosis a necessity (2, 36).

The best promising alternative to diagnose *T. vaginalis* will be molecular techniques. Recently, a number of PCR assays (42,43) have been developed for the detection of *T. vaginalis* and these assays have generally proved more sensitive than culture; however, PCR also requires a dedicated laboratory, sophisticated equipment, and specially trained laboratorians (5). Molecular diagnostic techniques enjoy high sensitivity and specificity when compared to conventional methods (41, 42).

In this study, a total of 1207 women of childbearing age were screened for trichomoniasis. Vaginal swabs were cultured and interpreted as described before. Culture is considered to be the reference method or the “gold standard” for diagnosing *T. vaginalis*.

In this study, 164 out of 1207 (13.6%) women screened for trichomoniasis were found to be infected with *T. vaginalis* by culture.
High infection rate was observed in Hebron (18.9 %) and Salfit (20 %), these two districts are located near major Israeli settlements in the West Bank. Palestinian men from these latter two districts working in Israeli territories may be the source of high infection rate.

Married women showed an infection rate of (13.6%). The infection rate of *T. vaginalis* was found to be the higher (20.7%) among young women of reproductive age (15 to 18 years old). Similar results were reported by various studies, in New York 13-25% of women where attending gynecology clinics were diagnosed for trichomoniasis (49), in Missouri, USA 15% of sexually active adolescents (50) and 12.9% in adolescent girls (51). A study done on women visiting STDs clinics in central North Carolina using PCR-based enzyme linked immunosorbent assay, the infection rate among these women was found to be 16.7% (52).

Another study on adolescent girls in Birmingham, Alabama, reported an infection rate of 12.9% (53). The reported results from these studies are similar to the results obtained by us. Another study on women visiting STDs clinics reported a higher infection rate of 40% (37). It is clear from the previous information that the infection rate of *T. vaginalis* depends on the patient population studied and the
diagnostic method used. Prostitutes have the highest infection rate of 50-70% (49).

It is apparent that disease occurrence correlates with the level of sexual activity of the group of women being studied. Sexually active women are at an increased risk for vaginitis because the presence of semen in the vagina may raise the pH and thereby allow for a proliferation of pathogenic anaerobic bacteria (17). However, the infection may also be acquired from toilet facilities, medical instruments or the exchange of underclothing. Other factors such as the susceptibility of the host, her physiological condition, the virulence and size of the inoculums, as well as social habits and hygiene conditions, might have an effect on the pathogenesis of these organisms (4, 6).

The infection rate of these pathogens was higher among camp residents for *T. vaginalis* (25.4%) than residents of rural and urban area (12.6-14.3%) (Table 2). This may be due to the lack of hygiene and crowded area of the camp.

Pregnant women showed an infection rate of 28.1%. A study on pregnant women done on inmates in New York City had reported a rate of infection of 47% (54). The reason for the high rate of
trichomoniasis in pregnant women can be attributed to the increased pelvic vascularity and elevated estrogen levels. Estrogen increases the rate of growth and maturation of the vaginal squamous epithelial cells (55). Estrogen can also increase the acidity (lower pH) of the vagina; the mechanism for lower pH is attributed to a general increase in metabolic activity under the influence of estrogen. The glycogen in the vaginal epithelium breaks down and results in increased lactic acid. The lower pH (3.5-6) is thought to ward off infection to some degree and alter the growth of pathogenic bacteria in the vagina (30).

Data obtained in this study support the role of contraceptives in reducing the rate of infection by *T. vaginalis*. It was found that women, who did not use contraceptives, had an infection rate of 17.1%. The infection rate among women using oral contraceptives was found to be 9.2%. This significant decrease in the rate of trichomoniasis may be due to the progestin component causing thickening of the cervical mucosa, which inhibits sperm and bacterial penetration (30, 56). The decreased duration of menstrual flow that accompanies the use of oral contraceptives theoretically also creates a shorter interval in which bacterial colonization may occur (30, 56). The infection rate when barrier contraceptives (condoms) were used
was 11.1%. Barrier contraceptives (condoms) has proven to be the most effective mechanical barrier to various microorganisms (56,57). Thus women using either barrier or oral contraceptives in the 6 months before pregnancy are far less likely to be colonized by *T. vaginalis* (58).

In this study, the infection rate of *T. vaginalis* in lactating women was found to be 11.4%. Although this result is somewhat lower than results obtained for married women (13.6%), it is significantly lower than the rate obtained for young women of reproductive age (20.7%).

The infection rate of *T. vaginalis* among Iraqi lactating women was found to be 6.5% which is much lower than our results (29).

Symptomatic vaginal trichomoniasis is extremely unusual in lactating patients, since the glycogen-poor atrophic vagina of the breastfeeding woman is relatively hostile to the trichomonad (30, 55).

The infection rate of *T. vaginalis* among women of different socioeconomic status and different educational levels was found to be 12.8% to 15.8%. These results are comparable to the results obtained for married women. These factors have no significant effect on the rate of trichomoniasis (57). Similar results were reported by study done in Basra, Iraq where no statistically significant differences in the
infection rate by *T. vaginalis* in relation to occupation, level of education or economic status was observed.

Traditionally, diagnosis of *T. vaginalis* was based on clinical signs and symptoms. Such symptoms include vaginal discharge, irritation, genital soreness, pelvic pain, burning sensation and vaginal pH. It should be emphasized here that 10-50% of women may be asymptomatic (19, 11, 36, 48).

The rate of infection with *T. vaginalis* in symptomatic women was comparable to that obtained for married women. The infection rate as related to the various symptoms and ranged from 13.1% to 15.9%. These results do not correlate any particular symptom with increased rate of trichomoniasis. Earlier studies done by Reine et al., 1990, correlated the symptoms with the wet mount identification method of the organism (49). Considering the lack of accuracy and decreased sensitivity (< 40%), for this method, these results can be questionable. Other studies reported that a pH greater than 4.5 was found in 80 to 90 % of patients with trichomoniasis (2, 10, 21).

In this study, 164 specimens out of 1207 attending reproductive health clinics were found to be positive by culture method. A total of 100 specimens were randomly selected for PCR analysis. The primer
set BTUB 9/2 was used to amplify a 112-bp fragment. Only 82 specimens (82%) of *T. vaginalis* were successfully detected by PCR giving the single predicted product of 112-bp in agarose gel electrophoresis.

Culture is considered to be the gold standard; with modified Diamond’s medium has high sensitivity of 92-95% and specificity near 100% (37). The disadvantage of the culture method is the cost, the time delay for a diagnosis (usually 2-7 days), and the need to delay treatment until the results are available. Patients will continue to be infectious to others during this time. In addition, trichomoniasis culture medium is not available to many physicians. Many studies were conducted to compare the diagnostic sensitivity of PCR and culture. One study was done on 337 women (59). PCR had the highest sensitivity for *T. vaginalis*, detecting 84% of samples as compared to 78% by culture method. Another study was conducted in Belgium to compare culture and different PCR methods (60). In the later study, there were variations in the different PCR assays depending on the primer set used. The prevalence was found to be 7.7% with culture as compared to 18.8% with PCR and gel electrophoresis.
It is apparent that PCR methods have high sensitivity and specificity with several advantages over other diagnostic techniques. PCR results can be available in 2 to 3 days with high sensitivity and it is cost effective when compared to the cost of culture.
4.1 Conclusion and Recommendation

1. In conclusion, this is the first study to be conducted on one of the most probably common sexually transmitted diseases in Palestine, trichomoniasis. The infection rate was found to be 13.6% which is mainly obtained from examining normal women attending reproductive health clinics.

2. Sexually transmitted diseases should be of public health concern in Palestine, therefore immediate intervention and rapid action by the Ministry of Health like surveillance for STDs is required in order to prevent and control such diseases.

3. Regarding prevention and treatment, both partners should receive careful examination and treatment once infection is suspected in either one of them.

4. Sterile medical examination instruments must be used at the clinic to prevent transmission of the disease.

5. Routine screening and providing suitable treatment for women with trichomoniasis before any reproductive tract surgery as well as before pregnancy should be done.
6. In case of infection of either the woman or her partner, barrier protection (condoms) should be used during intercourse until the infection is eradicated in both partners.

7. The population must be educated about this disease and the means of its transmission, since education on sexual behavior and genital hygiene may help in its prevention and control.
5. REFERENCES


4. Silva-Filho. How laminin-1 can be recognized by the protozoan parasite Tritrichomonas foetus: possible role played by the extracellular matrix glycoprotein in both cytoadhesion and cytotoxicity exerted by the parasite. Parasitol Int, 2002; 51:305-307.


60. Crucitti T, Van Dyck, Tehe E, Abdellati S, Vuylsteke B, Buve A and Laga M,. Comparison of culture and different PCR assays for detection of Trichomonas vaginalis in self collected vaginal swab specimens. Sex Transm Infect, 2003;79:393-398
6. Annexes

6.1 Annex (1)

Distribution of the sample in the different districts of West Bank

(according to the last national population count on 2003)

<table>
<thead>
<tr>
<th>District</th>
<th>Estimated population 2003</th>
<th>Sample size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hebron</td>
<td>505694</td>
<td>295</td>
</tr>
<tr>
<td>Bethlehem</td>
<td>169317</td>
<td>110</td>
</tr>
<tr>
<td>Jerusalem</td>
<td>144835</td>
<td>59</td>
</tr>
<tr>
<td>Ramallah</td>
<td>269827</td>
<td>158</td>
</tr>
<tr>
<td>Jericho</td>
<td>43545</td>
<td>40</td>
</tr>
<tr>
<td>Nablus</td>
<td>318240</td>
<td>186</td>
</tr>
<tr>
<td>Jenin</td>
<td>289841</td>
<td>169</td>
</tr>
<tr>
<td>Tulkarem</td>
<td>163397</td>
<td>95</td>
</tr>
<tr>
<td>Salfit</td>
<td>60130</td>
<td>35</td>
</tr>
<tr>
<td>Qalqilia</td>
<td>90729</td>
<td>53</td>
</tr>
<tr>
<td><strong>Total west Bank</strong></td>
<td><strong>2055555</strong></td>
<td><strong>1200</strong></td>
</tr>
</tbody>
</table>
6.2 Annex (2)

نموذج موافقة

صممت هذه الدراسة لمعرفة الوضع الصحي للمرأة، خاصة فيما يتعلق بأمراض الجهاز التناسلي التي قد تؤدي إلى مضاعفات ومشاكل منها التهاب الجهاز التناسلي أو العقم، أو مشاكل ومضاعفات في الحمل قد تنتقل إلى الجنين وتؤثر عليه، علمًا بأنه يمكن منع المضاعفات بالكشف المبكر ومعالجتها.

كافة النتائج ستبقى سرية ويبقى لكم خيار المشاركة في هذه الدراسة، كما يحق لكم طرح أي سؤال أو استفسار.

في حال الموافقة، ستؤخذ عينة دم ومسحة من المهبل لتم فحصها في مختبر الصحة العامة.

مع تقديرنا لتعاونكم ومشاركتكم لإنجاح الدراسة.

أنا الموقعة أدناه أوافق على المشاركة.

الاسم: __________________________

التوقيع: ________________________

التاريخ: __/__/
6.3 Annex (3)

نموذج (2)

استبيان

1- الرقم المتسلسل: (خاص بالمختبر)

2- اسم العيادة: 

3- التاريخ: ___/__/___

4- اسم المريضة: 

5- تاريخ الميلاد: ___/__/___

6- عدد الأطفال: 

7- الوضع الاجتماعي: 1- متزوجة 2- مطلقة 3- أرمل

8- المؤهل العلمي: 1- ابتدائي 2- إعدادي 3- ثانوي 4- جامعي
9- المؤهل العلمي للزوج: 1- ابتدائي 2- إعدادي 3- ثانوي 4- جامعي

10- العنوان:
1- مدينة 2- قرية 3- مخيم

11- معدل دخل الأسرة:

12- عمل الزوجة:

13- عمل الزوج:

14- هل تعملين في هذه الفترة أية وسيلة لمنع الحمل (في حال الإجابة بنعم، حددي)
1- نعم 2- لا

15- هل يستخدم الزوج العازل الذكري (في حال الإجابة بنعم، حددي)
1- نعم 2- لا

16- لتنظيم النسل 2- لتجنب انتقال الأمراض

( PH )

16- درجة الحموضة (PH):
الأعراض:

17- هل تعانين من احمرار في منطقة الجهاز التناسلي؟
1- نعم
2- لا

إذا كان الجواب "لا" انتقل إلى نقطة رقم (20)
إذا كان الجواب "نعم" اجب عن الأسئلة التالية:

18- هل هناك ألم؟
1- نعم
2- لا

19- منذ متى تعانين من الاحمرار؟ ________ أيام.

20- هل هناك إفرازات مهبلية؟
1- نعم
2- لا

إذا كان الجواب "لا" انتقل إلى نقطة رقم (23)
إذا كان الجواب "نعم" اجب عن الأسئلة التالية.

21- ما لون الإفرازات؟
1- أبيض
2- أصفر
3- أبيض مخضر
22 - هل راحتها كريهة؟

1- نعم  2- لا

23 - هل هناك حرقة عند التبول؟

1- نعم  2- لا

24 - هل هناك ألم في منطقة الرحم (أسفل البطن)؟

1- نعم  2- لا

25 - هل هناك ألم أثناء الجماع؟

1- نعم  2- لا