



Prevalence of *Trichomonas vaginalis* in Women Visiting 2 Obstetrics and Gynecology Clinics in Daegu, South Korea

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Abstract: This study explored epidemiological trends in trichomoniasis in Daegu, South Korea. Wet mount microscopy, PCR, and multiplex PCR were used to test for *Trichomonas vaginalis* in vaginal swab samples obtained from 621 women visiting 2 clinics in Daegu. Of the 621 women tested, microscopy detected *T. vaginalis* in 4 (0.6%) patients, PCR detected *T. vaginalis* in 19 (3.0%) patients, and multiplex PCR detected *T. vaginalis* in 12 (1.9%) patients. Testing via PCR demonstrated high sensitivity and high negative predictive value for *T. vaginalis*. Among the 19 women who tested positive for *T. vaginalis* according to PCR, 94.7% (18/19) reported vaginal signs and symptoms. Notably, more than 50% of *T. vaginalis* infections occurred in females younger than 30 years old, and 58% were unmarried. Multiplex PCR, which simultaneously detects pathogens from various sexually transmitted infections, revealed that 91.7% (11/12) of patients were infected with 2 or more pathogens. *Mycoplasma hominis* was the most prevalent co-infection pathogen with *T. vaginalis*, followed by *Ureaplasma urealyticum* and *Chlamydia trachomatis*. Our results indicate that PCR and multiplex PCR are the most sensitive tools for *T. vaginalis* diagnosis, rather than microscopy which has been routinely used to detect *T. vaginalis* infections in South Korea. Therefore, clinicians should take note of the high prevalence of *T. vaginalis* infections among adolescent and young women in order to prevent persistent infection and transmission of this disease.

Key words: *Trichomonas vaginalis*, trichomoniasis, PCR, multiplex PCR, multiple infection, sexually transmitted infection

Trichomoniasis, which is caused by the protist *Trichomonas vaginalis*, is one of the most common non-viral sexually transmitted infections, with an estimated prevalence of about 276 million new cases annually worldwide [1,2]. The prevalence of *T. vaginalis* varies and depends on the population, time, and region studied, but the rate is generally higher among women and older groups when compared to men or younger groups, which is in marked contrast to other sexually transmitted infections, such as *Chlamydia trachomatis* or *Neisseria gonorrhoeae* [3]. *T. vaginalis* infection causes vaginitis and cervicitis, and it is associated with serious complications, such as preterm birth, pelvic inflammatory disease, infertility, and an increased risk

of co-infection with human immunodeficiency virus [4-10]. However, more than 50% of *T. vaginalis* infections in women are asymptomatic and remain undiagnosed [11]. Thus, early diagnosis and treatment of *T. vaginalis* infections, especially asymptomatic infections, are imperative for women as this is an important public health concern.

It is also helpful to monitor changes in *T. vaginalis* infection rates in order to develop appropriate prevention measures. The most common clinical diagnostic test for *T. vaginalis* infections is microscopic evaluation of vaginal wet preparations; however, the sensitivity of wet mounts for detecting *T. vaginalis* is only 50-70% [12]. Cultures are the gold standard for diagnosing *T. vaginalis*, as cultures demonstrate increased sensitivity. However, they require at least a week for incubation, which results in a substantial delay between the patient's visit and final diagnosis [13,14]. Recently, nucleic acid amplification tests have been developed that use vaginal swabs, including PCR and transcription-mediated amplification. These have shown

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an improved sensitivity and specificity for the detection of *T. vaginalis* compared to microscopic examinations and culture, with sensitivity ranging from 64% to 100% [15,16]. Nucleic acid amplification tests also provide an improvement in medical screening for sexually transmitted infections. Recently, a new type of PCR, multiplex PCR, has been developed, which detects several different pathogens that may cause sexually transmitted diseases [17]. However, expensive equipment and technical training make nucleic acid amplification tests impractical for routine diagnosis in the clinical setting.

In this study, we examined 621 vaginal swabs from women attending obstetrics and gynecology clinics in Daegu, South Korea using wet mount microscopy, PCR, and multiplex PCR to determine the prevalence of *T. vaginalis* and associated sexually transmitted infections in *T. vaginalis*-positive women.

From May 2013 to November 2013, 621 vaginal swab samples were obtained from women visiting 2 obstetrics and gynecology clinics in Daegu, South Korea. These were screened for trichomoniasis by wet mount microscopy and PCR and were tested for bacterial sexually transmitted diseases by multiplex PCR. The study was approved by the Institutional Review Board of Kyungpook National University Hospital (IRB no. KNUH 2013-04-051). Each participant was asked to complete a written consent form to verify informed consent and to obtain permission to collect the specimen. Data about vaginal signs and symptoms, age, and current pregnancy status were obtained through self-report. Study questionnaires and samples were labeled with a unique surveillance number delinked from patient identifying information.

Wet mount microscopy was performed at the clinical sites within 10 min of sample collection. Observation of motile trichomonads was required to classify a wet mount as positive for *T. vaginalis*. The study questionnaires and samples were transported to our lab using eNaT™ nucleic acid transport collection tubes (Copan Flock Technologies, Brescia, Italy). The diagnosis of *T. vaginalis* infection was made using PCR that targeted Tvk [17] and the β -tubulin gene [18]. The frozen swab samples were thawed, suspended in PBS, and collected by centrifugation at 13,000 g for 10 min.

DNA from the cell pellets were extracted using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The PCR conditions were as follows: the initiation step was conducted at 95°C for 5 min, followed by 40 cycles at 90°C for 1 min, then 60°C for 30 sec, and 72°C for 2 min. A final extension step at 72°C was conducted

for 10 min. The PCR primer set Tvk 3/7 (Tvk 3, 5'-ATTGTCGAA-CATTGGICTTACCCIC-3'; Tvk 7, 5'-TCTGTGCCGCTTCAAG-TATGC-3') [17] amplified the 261 base pair (bp) products. The PCR primer set β -tubulin 9/2 (β -tubulin 9, 5'-CATTGATAAC-GAAGCTCTTACGA-3'; β -tubulin 2, 5'-GCATGTTGTGCCGGA-CATAACCAT-3') [18] amplified DNA products of 112 bp from *T. vaginalis* genomic DNA. PCR using the Tvk 3/7 primer set and multiplex PCR were performed in duplicate. If the results of the duplicate reactions of each test were not concordant, a third amplification and detection was performed to resolve the discrepancy. In cases of discrepancies between PCR and multiplex PCR results, PCR using the β -tubulin 9/2 primer set was performed, and these PCR results (both Tvk 3/7 and β -tubulin 9/2 PCR-positive) were considered final (positive for *T. vaginalis*).

The test sensitivities and specificities were determined using PCR as the gold standard. The percentage sensitivities and specificities with 95% confidence intervals were calculated with MedCalc version 7.0 (MedCalc Software bvba, Ostend, Belgium). Differences in the sensitivities and specificities were compared with McNemar's normal approximation test using SAS 9.3 (SAS Institute Inc., Cary, North Carolina, USA). The degree of agreement was determined using Kappa statistics in SAS 9.3 (SAS Institute Inc.). Results were considered statistically significance for *P*-values less than 0.001. In this study, the lower limit of *T. vaginalis* detection by PCR using Tvk 3/7 and β -tubulin 9/2 was found to be 1 organism per reaction, which was consistent with a previous report [18]. The presence of multiple infections with *T. vaginalis*, including *C. trachomatis*, *N. gonorrhoeae*, *Mycoplasma genitalium*, *Ureaplasma urealyticum*, and *Mycoplasma hominis* [19], were simultaneously detected using multiplex PCR (STD6 ACE Detection Kit, Seegene, Seoul, Korea).

A total of 621 subjects who visited 2 obstetrics and gynecology clinics in Daegu, South Korea were included in this study. The mean age of all patients was 36.2 years old (range 16-64 years), although the group was age-diverse (Table 1). The subjects included 199 (32%) single women and 422 (68%) married women; of these, 41 women were pregnant (6.6%) (Table 1). Among all 621 subjects, 557 women reported experiencing vaginal signs and symptoms, including vaginal discharge (473 women, 76.2%), itching (283 women, 45.6%), odor (199 women, 32.0%), dysuria (21 women, 3.38%), and abdominal pain (21 women, 3.4%) (Table 1).

Microscopic examination of wet mounts diagnosed *T. vagi-*

nalis in 4 (0.6%) women. PCR detected *T. vaginalis* in 19 (3.1%) women. *T. vaginalis* was detected via multiplex PCR in 12 (1.9%) women (Table 2). The PCR results (19 positives and 602 negatives) were used as the gold standard for detecting *T. vaginalis*, and as such, the PCR results were used as a baseline to assess the accuracy of the other diagnostic tests. Accordingly, the sensitivity, specificity, positive predictive value, and negative predictive value were calculated for each test.

Microscopic examinations were positive for 4 specimens, resulting in a sensitivity of 15.8% (95% CI, 4.2-40.5%) and a specificity of 99.8% (95% CI, 98.9-100%) (Table 2). All 4 positive specimens detected by microscopy were confirmed via PCR and multiplex PCR. The results obtained by multiplex PCR were positive for 12 specimens, resulting in a sensitivity of 57.9% (95% CI, 79.1-100%) and a specificity of 99.8% (95% CI, 49.2-75.3%) (Table 2). All 12 *T. vaginalis*-positive

specimens detected by multiplex PCR were confirmed using PCR. In addition, 7 of the 19 samples found to be positive for *T. vaginalis* using PCR (7/19, 36.4%) were negative according to multiplex PCR and thus considered to be false negatives. Microscopy and multiplex PCR both showed high specificity (99.8%, 95% CI: 98.9-100%). However, sensitivity was distinct between the 2 methods. Compared to microscopic examination, multiplex PCR demonstrated a significantly higher sensitivity ($P < 0.001$ vs microscopy) and better agreement (concordance, 94.9%; kappa value, 0.865) with PCR than microscopy (Table 2).

These findings were consistent with previous reports in which the newly developed nucleic acid amplification tests, such as PCR and multiplex PCR, were able to accurately detect more samples infected with *T. vaginalis* compared to traditional techniques, including microscopic examination of wet mounts and cultures [18-20]. This result is noteworthy since misdiagnosis of *T. vaginalis* as a result of using a less accurate diagnostic method would result in a persistent infectious state, and may subsequently lead to serious complications as well as possibly broad transmission of the infection. In addition, administration of metronidazole is an effective treatment for *T. vaginalis* infection; therefore, diagnosis by a highly sensitive method followed by rapid treatment would be a good strategy to control this disease.

In this cohort of female patients who visited obstetrics and gynecology clinics, the prevalence of *T. vaginalis* infection was 3.1% (19/621) according to PCR testing. To date, several studies regarding *T. vaginalis* infection in South Korea have reported varying prevalences of *T. vaginalis* according to the specific investigators and study populations, as reviewed by Ryu and Min [21]. Similarly, the prevalence of *T. vaginalis* infection showed variability in 2 recent studies, with a rate of 2.6% in Cheonan and 0.1% in Seoul, South Korea [17,22]. Our results showed an infection rate of 3.0% (19/621), which is similar to the rate found by the surveillance study performed in Cheonan. This is likely due to the fact that our research in Daegu and

Table 1. Analysis of *Trichomonas vaginalis* infection status according to sociodemographic characteristics and urogenital symptoms (N=621)

Factor	Characteristic	No. of patients	<i>T. vaginalis</i> prevalence (%)
Overall		621	19 (3.1)
Age (yr)	<20	14	2 (14.3) ^a
	20-29	197	8 (4.1) ^a
	30-39	171	3 (1.8)
	40-49	157	4 (2.5)
	50-59	63	2 (3.2)
	60-69	19	0 (0.0)
Marital status	Single	188	10 (5.3) ^a
	Married	422	8 (1.9)
	Divorced	1	0 (0.0)
	Missing	9	1 (11.1)
Pregnancy status	Yes	11	2 (18.2)
	No	74	14 (18.9)
	Missing	16	3 (18.8)
Urogenital symptoms	None	64	1 (1.6)
	Vaginal discharge	473	14 (3.0)
	Vaginal itching	283	9 (3.2)
	Vaginal odor	199	10 (5.0)
	Dysuria	21	2 (9.5)
	Lower abdominal pain	21	0 (0.0)

^a $P < 0.05$.

Table 2. Comparison of results according to diagnostic method among 621 specimens

Assay	No. positive	No. negative	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	% agreement with nested PCR (Kappa)
Microscopy	4	617	15.8 (4.2-40.5)	99.8 (98.9-100)	75.0 (21.9-98.7)	97.4 (95.7-98.5)	81.9 (0.624)
PCR	19	602					
Multiplex PCR	12	609	57.9 (79.1-100)	99.8 (98.9-100)	91.7 (59.8-99.6)	98.7 (97.3-99.4)	94.9 (0.865)

PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval.

the study conducted in Cheonan both assessed samples from symptomatic patients who visited the hospital, while the study in Seoul assessed samples from healthy women who visited the hospital for a routine health checkup.

The median age of the 19 females with *T. vaginalis*-positive specimens was 31.9 years (range 16-53 years). As shown in Table 1, peak *T. vaginalis* infection incidence was found in the younger study group (<30 years). Notably, 10 (52.6%) of the 19 *T. vaginalis*-infected subjects were under 30 years old, and the positive rate in the age group <30 years (4.8%, 10/211) was higher than the rate in the age group over age 30 (2.2%, 9/410) ($P < 0.05$). A similar tendency was reported in past studies, as reviewed by Ryu and Min [21], in that patients between 20-34 years old were most affected by *T. vaginalis* among patients of all ages who visited obstetrics and gynecology practices. However, these results are inconsistent with recent studies conducted in other countries in which the incidence of *T. vaginalis* increased by age [23,24]. The high positive rate in the younger study group (<20 years) might be a result of the social atmosphere in South Korea in which young women often feel uncomfortable about going to obstetrics and gynecology clinics and thereby neglect the disease until vaginal symptoms and signs become serious. Since *T. vaginalis* infections may become persistent without treatment, this tendency to avoid care should be considered, as early diagnosis and treatment at a young age are necessary to prevent the transmission of this disease.

An examination of marital status showed that 5.3% (10/188) of *T. vaginalis*-positive patients were single, and 1.9% (8/422) were currently or previously married, demonstrating that *T. vaginalis* is more prevalent in single women ($P < 0.05$). *T. vaginalis* infection in this cohort was more prevalent among adolescent and younger unmarried women. According to an analysis of the self-administered questionnaires, only 1 patient reported no symptoms among the 19 patients who were positive for *T. vaginalis*. On the other hand, 94.7% (18/19) of *T. vaginalis*-infected female patients reported urogenital symptoms, such as vaginal discharge (73.7%, 14/19), vaginal itching (47.4%, 9/19), odor (52.6%, 10/19), and dysuria (10.5%, 2/19).

According to multiplex PCR tests for simultaneous detection of pathogens that cause sexually transmitted infections, *T. vaginalis* was detected in 12 out of 621 samples (1.9%) (Table 3). As shown in Table 2, multiplex PCR was less sensitive for *T. vaginalis* detection than PCR (which detected 19 positive samples). While previous reports have suggested possible limitations of multiplex PCR, which include PCR drift by stochastic

Table 3. Frequency of comorbid sexually transmitted infections in *Trichomonas vaginalis*-positive samples according to multiplex PCR

Pathogens	No. patients	Percentage (%)
Single infection with <i>T. vaginalis</i>	1	8.3
Double infection		
<i>T. vaginalis</i> - <i>U. urealyticum</i>	2	16.7
<i>T. vaginalis</i> - <i>M. hominis</i>	1	8.3
<i>T. vaginalis</i> - <i>N. gonorrhoeae</i>	1	8.3
Triple infection		
<i>T. vaginalis</i> - <i>M. hominis</i> - <i>U. urealyticum</i>	4	33.4
<i>T. vaginalis</i> - <i>M. hominis</i> - <i>C. trachomatis</i>	3	25.0
Total	12	100.0

fluctuation due to the interaction of PCR reagents or by competitive inhibition from PCR selection [25], multiplex PCR is useful when detecting comorbid pathogens simultaneously, demonstrating both high sensitivity and specificity [26].

T. vaginalis is frequently co-localized with bacterial vaginosis, which is an established risk factor for adverse reproductive outcomes, such as pregnancy loss, premature rupture of membranes, and preterm labor [4,27]. Among the 12 *T. vaginalis*-positive samples detected by multiplex PCR, 91.7% (11/12) were positive for co-infection. Moreover, 7 (58.3%) of the samples were positive for triple infections, which is defined as being infected with *T. vaginalis* and 2 different pathogens among the 5 pathogens (*C. trachomatis*, *N. gonorrhoeae*, *M. genitalium*, *U. urealyticum*, and *M. hominis*). Four (33.3%) samples were positive for double infections, defined as being infected with *T. vaginalis* and 1 pathogen among those 5 pathogens. As shown in Table 3, *M. hominis* (66.7%, 8/12 samples) was the most prevalent co-infection pathogen, followed by *U. urealyticum* (50%, 6/12), and *C. trachomatis* (25%, 3/12). *T. vaginalis* infection in conjunction with both *M. hominis* and *U. urealyticum* was the most common triple infections (33.3%, 4/12). Since these pathogens have a common route of transmission, i.e., via the vagina, concurrent sexually transmitted infections, including *M. hominis*, *U. urealyticum*, *C. trachomatis*, and *N. gonorrhoeae*, are frequently detected in persons with *T. vaginalis* infection [28,29].

Comorbid infection with these pathogens may be more prevalent among female *T. vaginalis*-positive patients due to their symbiotic association with *T. vaginalis*, as previously suggested. In the present study, co-infections with *T. vaginalis* (both double as well as triple infections) were observed in 11 out of 12 *T. vaginalis*-positive samples diagnosed by multiplex

PCR. Consistent with the high prevalence of *T. vaginalis* infection in young women, 6 (50%) of the 12 *T. vaginalis*-positive patients that were detected by multiplex PCR assay were aged <30 years, which shows that there was a high percentage of co-infection with other sexually transmitted infections. Moreover, our previous surveillance studies of male patients attending a primary care urology clinic in the same region (Daegu) and time period found a *T. vaginalis* prevalence of 4% (8/201), which was slightly higher than the rate among female patients [30]. However, *T. vaginalis* was significantly more prevalent among older men (≥ 40 years, 87.5%) than among relatively younger men (<40 years, 11.5%), which is opposite of the distribution pattern of *T. vaginalis* prevalence in women by age. A limitation of the present study is the relatively small sample size, therefore providing only limited surveillance results. Nevertheless, these results provide good evidence for a bipolarized distribution of the prevalence of *T. vaginalis* infection according to age between male and female patients.

In this study, the prevalence of *T. vaginalis* infection in Daegu, South Korea was evaluated after determining the efficacy of 3 diagnostic methods for detecting *T. vaginalis*. Infection with *T. vaginalis* was detected in 3.1% of the study population in Daegu via PCR. Interestingly, a higher infection rate was observed in the younger study group (<30 years), and a high percentage of the total study population was co-infected with other sexually transmitted infections. Further studies are required to monitor the prevalence of *T. vaginalis* in adolescents and young women to prevent the transmission and burden from this disease.

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CONFLICT OF INTEREST

We have no conflict of interest related to this work.

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