Prevalence of *Trichomonas vaginalis* Based on Clinical Manifestation and Polymerase Chain Reaction among Reproductive Women

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Abstract

Objective: To measure the prevalence of *Trichomonas vaginalis* (*T. vaginalis*) based on clinical manifestations and polymerase chain reaction (PCR) among reproductive women.

Methods: Subjects of the study were the vaginal swab obtained from reproductive women who attended the gynecology examination at Kandanghaur and Sindang primary health care of Indramayu District, West Java in 2016. This study was a descriptive study with cross-sectional method. Sampling was performed with total sampling method and 76 of vaginal swabs were included in this study. The prevalence of T. vaginalis was measured using PCR. The vaginal specimens were collected and then processed for PCR analysis using TVK3/TVK7.

Results: Prevalence of *T. vaginalis* among reproductive women in Indramayu District, West Java that analyzed using PCR was 0%. This result could be affected by the study setting in community, presence or absence of symptoms, and population studied.

Conclusions: There were no positive results of *T. vaginalis*, suggested by the samples that obtained from community-based of a low-risked population.

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Introduction

Trichomonas vaginalis (*T. vaginalis*) is a flagellated protozoa known as the most common human parasite that responsible for sexually transmitted infection (STI) in the world.[1–5] World Health Organization (WHO) estimated that there were 170–190 million cases of *T. vaginalis* infection worldwide each year and nearly 90% of these infections occur in resource-limited settings country.[1,2,4] According to WHO estimation, the prevalence of *T. vaginalis* infection in women between the ages of 15 and 49 in 2008 were 22% in America, 20.2% in Africa, 8% in Eastern Mediterranean, 5.8% in Europe, 5.7% in Western Pacific, and 5.6% in Southeast Asia.[6] The prevalence of the *T. vaginalis* infection in Indonesia had not been clearly studied. However, a study showed that the prevalence of *T. vaginalis* among female workers in Kupang, Nusa Tenggara Timur Province was 5%.[7]

The trichomonas vaginalis infection or trichomoniasis among women has been associated with mild to severe reproductive health outcomes including vaginitis, cervicitis, urethritis, low birth weight, premature rupture of membranes, pre-term delivery, and pelvic inflammatory disease.[3,4] Trichomoniasis also have several serious complications

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including increased risk of HIV acquisition, increased risk of cervical cancer, and shedding of herpes simplex virus-2 (HSV-2) in the genital tract of women, which could result in increased transmission of other STIs.[1,3,4] While 73% of women infected with T. vaginalis are asymptomatic, one-third of them became symptomatic within six months.[3] In women who have symptoms, usually the symptoms are similar to other STIs, include vaginal discharge which is often malodorous and yellow-green colour, dysuria, itching, vulvar irritation, and abdominal pain.[1,8] However, trichomoniasis has specific symptoms that only occurred in 2–5% of women, known as "strawberry cervix", as well as frothy vaginal discharge in 12% of women with trichomoniasis.[8]

There are several methods to identify *T. vaginalis* include wet mount microscopy, culture, and nucleic acid amplifications Tests (NAATs).[9] Wet mount microscopy method has low sensitivity, which ranges from 44% to 68% and requires trained microscopist.[10] The sensitivity can dramatically reduce if there was a delay as short as 10–30 minutes between collection and microscopic examination.[10] Meanwhile, culture method provides sensitivity that ranges from 44–75% but needs daily examination by a trained microscopist and the final result may take up to a week.[10] Both wet mount microscopy and culture method, require specimen handling, processing, and transport conditions to preserve viable, motile organism.[10] In contrast with NAATs method, polymerase chain reaction (PCR) is one of the NAATs method using replication and amplification technique from specific individual deoxyribonucleic acid (DNA) target sequences. Thus, the sensitivity of NAATs is inherently greater than other methods ranges from 76–100%.[10] Primer sets TVK3/TVK7 known as the most sensitive primer among others primer sets to identify *T. vaginalis* because the target of this primer is a repetitive DNA fragment that improve the detection level and produce positive results on specimens from asymptomatic women.[11]

The prevalence of *T. vaginalis* has been found to vary according to geographical location, study setting (sexual health clinic or community setting), the presence or absence of symptoms, population studied (ethnic group, age, and sex), and the diagnostic techniques used.[7] This study identified *T. vaginalis* from reproductive women using vaginal swab as the specimen and PCR method with TVK3/TVK7 as the primer sets.

Methods

This was a descriptive study with cross-sectional method. Subjects of the study were the vaginal swab obtained from reproductive women who attended gynecology examination at Kandanghaur and Sindang primary health care of Indramayu District, West Java, Indonesia, in 2016 by total sampling.

Inclusion criteria for this study were vaginal swab from women with positive visual inspection with acetic acid (VIA) test, or women with vaginal discharge, or women with vaginal discharge with itching, or women with malodorous vaginal discharge. Exclusion criteria was the volume of the vaginal swab which less than 5µl.

The vaginal swab had already been obtained in year 2016 and stored in -20 ^{0}C for long-term examination. Therefore, samples used in this study were stored biological material, thus the informed consent was unnecessary. The extracted genomic DNA samples underwent PCR and electrophoresis at the Microbiology and Molecular Laboratory of Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia. The PCR kit consist of, primer sets TVK3/TVK7 and KAPA Taq Extra HotStart ReadyMix PCR kit®. The electrophoresis kit consist of peqGREEN DNA and RNA Dye®.

The PCR master mix was made according to the procedures provided by manufacturer. The initial reaction for PCR was performed at 95 ^{0}C for 5 minutes and then repeated 35 cycles of 30 sec at 95 ^{0}C, 30 sec at 57.4 ^{0}C, 2 minutes at 72 ^{0}C, finally additional extensions were done at 72 ^{0}C for 7 minutes and hold at 4 ^{0}C.

Upon completion of PCR, an aliquot was analyzed by electrophoresis in a 3% agarose gel in TAE buffer. The gel was stained with peqGREEN DNA and RNA dye® and was photographed under short ultraviolet light. The primer sets TVK3/TVK7 specifically amplify a 300 bp fragment of *T. vaginalis* genome.[11] The size of amplified products was assessed by comparison with a commercial weight marker (1Kb ladder).

Positive and negative controls were included in all PCR runs. The DNA extract from motile *T. vaginalis* in wet mount examination was used as a positive control. The negative control consisted of PCR master mix without DNA. This study was approved through the ethical clearance number 595/UN6.KEP/EC/2018 by the Health Research Ethic Committee, Faculty of Medicine, Universitas Padjadjaran, Bandung.

Table 1 Characteristics of the Subjects

Characteristics	Frequency (n)	PCR Positive of *T. vaginalis*	
		Frequency (n)	Percent (%)
Age			
20–29 years old	9	0	0
30–39 years old	42	0	0
40–49 years old	24	0	0
50–59 years old	1	0	0
Marital status			
Yes	76	0	0
No	0	0	0
Obstetrical status			
P0A0	5	0	0
P1A0	23	0	0
P1A1	3	0	0
P1A3	1	0	0
P2A0	17	0	0
P2A1	7	0	0
P3A0	9	0	0
P3A2	2	0	0
P4A0	3	0	0
P4A1	1	0	0
P4A2	1	0	0
P4A4	1	0	0
P5A0	1	0	0
P5A1	1	0	0
Education level			
Uneducated	2	0	0
Elementary school	28	0	0
Junior high school	24	0	0
Senior high school	16	0	0
Diploma	2	0	0
Bachelor	4	0	0
Occupation			
Housewife	68	0	0
Farmer	5	0	0
Government officer	1	0	0
Midwifery	1	0	0
Nurse	1	0	0

Results

During the gynecology examination at Kandanghaur and Sindang primary healthcare in 2016, from 147 women attended, 143 vaginal swabs were obtained, and 76 samples were stored in laboratory and underwent PCR examination for this study, while the rest of the samples could not be extracted because of the volume less than 5µl. The characteristics of subjects which vaginal swab underwent PCR were described (Table 1). The prevalence of *T. vaginalis* based on PCR were shown. Based on these data, the PCR results of *T. vaginalis* were negative in all samples.

The prevalence of *T. vaginalis* based on clinical manifestation was shown (Table 2). From this data, in some subjects who had one symptom or more, the PCR results were negative for *T. vaginalis*.

Result of electrophoresis was revealed (Fig. a–b). In all of the electrophoresis results, there was the positive control that has length 300kbp appropriate for the size of the DNA that amplified by TVK/TVK7 primer sets, but none of the samples have a positive result because there was no band appeared in all of the results.

Discussion

The prevalence of trichomoniasis reported in this study was 0% using PCR method (Fig. a–b). The socio-demographic characteristics and clinical manifestation of the study population may affect this finding.[12] The socio-demographic characteristics of the study population are portrayed (Table 1). Among 76 women who examined for *T. vaginalis*,

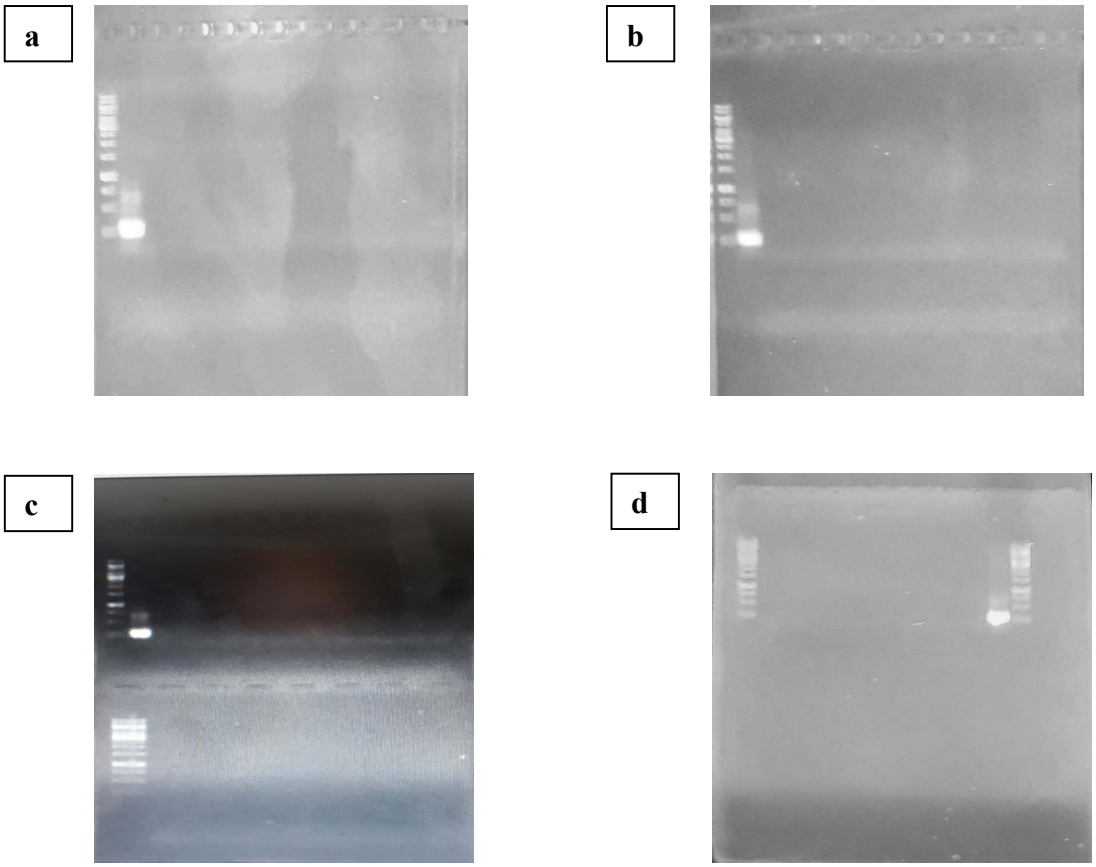

Fig. Electrophoresis Sample 1–12 (a), Electrophoresis Sample 13–24 (b), Electrophoresis Sample 25–28 (c), Electrophoresis Sample 65–76

Table 2 Prevalence of *T. vaginalis* Based on Clinical Manifestation

Characteristics	Frequency (n)	PCR Positive of T. vaginalis	
		Frequency (n)	Percent (%)
Vaginal bleeding			
Yes	1	0	0
No	75	0	0
Vaginal discharge			
Yes	24	0	0
No	52	0	0
Vaginal discharge with itching			
Yes	19	0	0
No	57	0	0
Malodorous vaginal discharge			
Yes	10	0	0
No	66	0	0
VIA test			
Positive	15	0	0
Negative	61	0	0

42 women in this study belonged to the age group of 30–39 years, but none of them were positive for *T. vaginalis* infection. This result was similar to several previous studies which majority of infected women were between the ages of 35–40 years, 26–35 years (37.93%), and 21–45 years (76%), respectively.[6,8,12] Unlike other nonviral STIs, trichomoniasis does not primarily reach young women (15–25 years old). It affects women during reproductive years, and high rates of infection are found in women between the ages of 35 and 40. Predisposing factors comprise of older age, use of oral contraceptives, trading sex, smoking, single marital status, and low socioeconomic class.[6]

Other socio-demographic factors for trichomoniasis are marital status and obstetrical status. All women in this study have the same marital status, while obstetrical status varied and the result was 17 women (22.36%) had history of abortion. This result was in contrast to a study which reported women who have abortion previously will have a higher prevalence of *T. vaginalis* infection.[13]

Both occupation and education level are predisposing factors for trichomoniasis in published literature. In the previous study, majority of those with trichomoniasis were unemployed and has less education level.[12] However, in this study, there was no difference between the occupation and education level with *T. vaginalis* infection.

The overall prevalence of *T. vaginalis* identification using PCR method was 0%. A previous study identified *T. vaginalis* with the same method as in this study which found the prevalence of the infection was 30%.[8] The negative findings of *T. vaginalis* in this study could be affected by samples that obtained from low-risked women and the study setting was community-based study. In contrary, in clinic-based studies there was a higher prevalence than community-based studies. In community-based study, the prevalence was 1% and 0.6% respectively. While in clinic-based study, the prevalence was 4.4% and 4.2% respectively.[14] Another risk factor associated with trichomoniasis was the last sexual intercourse. Therefore, the normal incubation period for trichomoniasis is 4–28 days. If the patients are infected by trichomoniasis, the patients are expected to be symptomatic within this period or immediately after. This may explain the lower risk of finding in those who had sexual intercourse over 30 days in

advance.[12] In this study, there were no data about last sexual intercourse.

The clinical manifestations varied among the 76 women, 52 women had no vaginal discharge, 57 women had no vaginal discharge with itching, 66 had no malodorous vaginal discharge, and 61 women had no cervicitis, indicated by negative VIA test. Most of women are asymptomatic but none of them had positive result for *T. vaginalis*. It is very difficult to predict the *T. vaginalis* infection only on clinical findings, compare to previous study in Belgium, 50% of the patient had normal vaginal findings and 20% of them were positive for trichomoniasis. While 36% of the patient had cervicitis and 44% of them were positive for trichomoniasis.[8]

This study was used PCR method to detect *T. vaginalis* since it offers an advantage of extreme sensitivity and ability to detect nonviable organisms.[8] Since most cases of trichomoniasis are asymptomatic and the infection could cause serious complication such as HIV, cervical cancer, HSV-2, and other STIs.[1,3,4] Thus, it is important to screen *T. vaginalis* infection in low-risked women to prevent it complications.

Moreover, diagnosis of trichomoniasis cannot be made solely on the basis of clinical presentation because the clinical symptom may be synonymous with those of other STIs, the specific symptom like strawberry cervix is seen approximately 2% of patients, and frothy discharge is seen only in 12% of women with trichomoniasis. It has been demonstrated that if these classical features are used alone in the diagnosis of trichomoniasis, 88% of these cases will not be diagnosed and 29% of uninfected women will be falsely indicated as having *T. vaginalis* infection. This suggests that clinical manifestation are not reliable diagnostic parameters and hence laboratory diagnosis is necessary for early and accurate diagnosis.[8]

This study had limitations such as the examination only covered the low-risked women and the study settings was a community-based study. Therefore, a further study can be conducted by using a broader population including high-risk subject.

In conclusion, the prevalence of *T. vaginalis* based on clinical manifestation and PCR among reproductive women in this study was 0%. However, the samples that obtained and stored from 2 years ago have a limited possibility to become contaminated because the samples already been extracted into DNA and stored in -20 ^{0}C. It was proven from the positive control that appeared in each electrophoresis results. The positive control used in this study was also obtained 2 years ago from motile *T. vaginalis* that underwent DNA extraction and stored together with other samples.

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