Prevalence of *Trichomonas vaginalis* in vaginal swabs from
sex workers in Angeles City, Pampanga, Philippines as detected by PCR

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Abstract: A prevalence study was conducted on *Trichomonas vaginalis* infection among female sex workers attending the Reproductive Health and Wellness Center of Angeles City, Pampanga in the Central Luzon region of the Philippines. Polymerase chain reaction (PCR) using *T. vaginalis*-specific primers TV3/7 was utilized to detect *T. vaginalis* in vaginal swabs from the study population. The lower limit sensitivity of *T. vaginalis* detection by the PCR assay was found to be one trichomonad. The overall prevalence in 377 women was 9.55%. More than half of the study subjects are 23-27 years old. However, the largest proportion of positive cases was found among subjects 18-22 years old, making it the age group with the highest *T. vaginalis* prevalence (12.84%).

Keywords: *Trichomonas vaginalis*, female sex workers, PCR, Philippines

INTRODUCTION

Trichomoniasis, caused by the protozoan parasite *Trichomonas vaginalis*, is one of the most common nonviral sexually transmitted infections (STI) in the world [1]. *T. vaginalis* infection may lead to vaginitis, urethritis, cervicitis, infertility, post-hysterectomy infection and pregnancy complications such as premature labor and low-weight offspring [2, 3]. The parasite also causes gonococcal urethritis, prostatitis, and perhaps other lower genitourinary tract syndromes in infected men [4]. Trichomoniasis has also been shown to be a risk factor for pelvic inflammatory disease [5] as well as transmission and infection with human immunodeficiency virus (HIV) [6]. Concomitant infection with other urethral pathogens such as *Neisseria gonorrhoea* and/or *Chlamydia trachomatis* has also been found to be common in men and women with trichomoniasis [7].

The estimated global incidence of *T. vaginalis* infections is over 170 million cases per year [6]. An estimated 7.4 million new cases of *T. vaginalis* infection occurred in 2000 in the United States, compared to 2.8 million cases of *C. trachomatis* infection and 718,000 cases of *N. gonorrhoea* infection [8]. Although trichomoniasis and other STIs have also been reported from Southeast Asia [9], data on trichomoniasis cases in most of the countries in the region are lacking. In the Philippines, female sex workers are prone to high rates of infection with *T. vaginalis*. Using the medium described by Feinberg and Whittington in 1957 [10], it was established in a study in 1986 that after incubation of cultures of vaginal swabs for 3 to 5 days, 42% of the female sex workers in Angeles City were positive for *T. vaginalis* infection [11]. More recently, Queza (2008) reported 6.81% of trichomoniasis cases among 969 women from different areas in the Philippines [12]. Various socio-demographic factors such as race, age, histories of previous STI, access to care, and personal health practices have been correlated with the presence of *T. vaginalis* and may be used to predict infection. The lack of specific guidelines for *T. vaginalis* screening in women may be due to the absence of epidemiologic data on *T. vaginalis* infection in many areas in the Philippines. Therefore, knowledge of *T. vaginalis* infection, distribution and prevalence in high-risk populations is necessary to evaluate the need for preventive measures and more sensitive diagnostic methods. In recent years, no population-based prevalence study on *T. vaginalis* infection has been conducted in Angeles City, Pampanga, Philippines. For this reason, we conducted the present study to estimate the prevalence of *T. vaginalis* infection in the city. *T. vaginalis* infection was detected by polymerase chain reaction (PCR), a method which has been found to be relatively sensitive and is now readily used for the detection and screening of other STI-causing pathogens such as *N. gonor-

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rhea and C. trachomatis. In this study, T. vaginalis-specific primers TV3/7 were used. The results of this study are essential to the development of preventive strategies.

MATERIALS AND METHODS

Study area

This study was conducted in Angeles City, Pampanga, located in the Central Luzon region of the Philippines (Figure 1). Angeles City is locally classified as a first-class highly urbanized city. The northwestern part of the city is the well-known Clark Freeport Zone, the former Clark Air Base and site of the largest United States (US) air base outside the continental United States of America. During the American colonial period, a large number of Filipino mestizos (people of mixed parentage, mainly American and Filipino) were born in the city. Today, Angeles City and Clark form a center for business, industry, aviation and tourism as well as a popular entertainment and gaming area in Central Luzon. However, since the early days of the Clark Air Base, prostitution has also been prevalent in many areas in the city especially those frequented by American servicemen and foreign tourists.

Angeles City is one of the foremost cities in the country serving as an HIV/AIDS sentinel site. The city has a Reproductive Health and Wellness Center (RHWC) which provides care and support programs for people with HIV/AIDS and serves as a resource center for STI/HIV/AIDS-related concerns.

Collection of specimens

Sample size estimation was determined using the statistical formula of Jones et al. (2003) [13], that is, assuming 0.05 level of significance $\alpha$ and 37% trichomoniasis prevalence in the Philippines [11]. The minimum calculated sample size for the study was 377. Therefore, vaginal swabs were acquired consecutively from 400 female sex workers undergoing weekly medical check-ups at RHWC. Informed consent was obtained from all study participants, and the study was approved by the Ethics Review Committee of the Office of the City Health Officer, Angeles City, Pampanga. Due to cross-contamination during collection and transport, only 377 of the 400 samples collected were processed. Each vaginal swab was placed in a sterile screw-capped tube containing 2 mL phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 8 mM Na$_2$HPO$_4$, 1 mM

![Figure 1. Map of the Philippines showing the location of the study area, Angeles City, Pampanga](image_url)
KH₂PO₄, pH 7.2) for DNA extraction.

**DNA extraction and PCR**

The vaginal swab samples in PBS were transported on ice to the Molecular Protozoology Laboratory of the Natural Sciences Research Institute, University of the Philippines. Approximately 1.5 mL aliquot of each sample was centrifuged at 10,000 rpm for 2 minutes, and the supernatant was discarded. The pellet was suspended in 75 µL sterile distilled water and subjected to genomic DNA extraction by the Chelex method [14]. Briefly, each cell suspension was supplemented with 200 µL of a 5% suspension of chelating resin, Chelex (Sigma) in PCR-grade water and incubated at 56°C for 20 minutes. The preparation was then mixed vigorously and boiled for 8 minutes. DNA was separated from Chelex beads by centrifugation at 10,000 rpm for 2 minutes after which the supernatant was transferred to a different microfuge tube and stored at -20°C until use.

PCR assays were performed by batches in an automated thermocycler (Techne). PCR using oligonucleotide primers, TV3 and TV7 was performed on all samples. This primer set specifically amplifies a 312-bp sequence from repetitive DNA in the *T. vaginalis* genome [15, 16]. Reactions consisted of 2U Taq DNA polymerase, 1x PCR buffer (MgCl₂ free), 2 mM MgCl₂, 0.2 mM of each dNTP, 10 µL of DNA template, and 1 µM of each primer (TV3 and TV7) with PCR-grade water to make up a final volume of 25 µL. Positive and negative controls were included in all PCR runs. The positive control consisted of DNA of *T. vaginalis* isolate from a previous vaginal swab sample collection from patients attending the Social Hygiene Clinic in Davao City, Philippines [12], while the negative control consisted of PCR mix with primers but no DNA template. PCR was performed with 1 cycle of 95°C for 5 minutes; 35 cycles of 90°C for 1 minute, 60°C for 30 seconds, and 70°C for 2 minutes, and a final extension step of 72°C for 7 minutes.

Dilutions were made from a live culture of a clinical isolate of *T. vaginalis* and tested to detect the lower limit sensitivity of the PCR assay. Dilutions corresponding to 1, 5, 10, and 100 trichomonads were made in deionized water, and then DNA was extracted and processed as described above.

**Agarose gel electrophoresis**

An aliquot (7 µL) of each of the PCR products was run in 1.5% agarose gels in 1x TAE (Tris-acetate-EDTA, 0.04 M Tris-acetate, 0.001 M EDTA) buffer at 100V. The gels were then stained with 0.5 µg/mL ethidium bromide, viewed under UV illumination, and photographed. The sizes of the amplified products were assessed by comparison with a commercial 100-bp weight marker (Roche Diagnostics, Inc.). Samples containing 300-bp fragments were considered positive for *T. vaginalis*.

**RESULTS**

In the present study, all of the 400 study subjects willingly participated in the study. The median age of the study population was 24.56 (range, 18-44). The largest portion of the study population was comprised of patients 23-27 years of age (51.59%), followed by ages 18-22 (28.84%), 28-32 (16.40%), 33-37 (2.65%), and 43-47 (0.27%). There were no study participants in the 38-42 age range (Figure 2).

All personal identifiers had been removed from samples and new, unique identifiers (sample codes 1-400) were assigned. A total of 377 samples were processed for PCR using primers TV3/7. Of the 377 clinical samples tested, 36 (9.55%) were positive for *T. vaginalis* infection. Figure 3 represents the agarose gel electrophoresis of some of the *T. vaginalis*-positive samples detected by the PCR. The lower limit sensitivity of *T. vaginalis* detection by the PCR assay was found to be one trichomonad (Figure 4).

Figure 5 shows the prevalence of trichomoniasis for each age group. To calculate the trichomoniasis prevalence, the number of positive cases within each age group was divided by the total number of participants corresponding to the group. As a result, the highest prevalence of *T. vaginalis* infection was found in the age group 18-22 (12.84%),
followed by 28-32 (9.6%) and 23-27 years old (8.2%). No positive cases were identified in the 33-37 and 43-47 age groups.

**DISCUSSION**

Constant screening of high-frequency transmitter groups such as female sex workers is an important approach in controlling the spread of STIs such as trichomoniasis. Female sex workers and their male clients are thought to act as core and bridging populations in the epidemiology of STIs [17]. The RHWC functions under the City Health Office of Angeles City as the only reproductive health clinic regularly monitoring the city’s entertainment workers. Sex workers from different parts of the city visit the clinic for a weekly smear check-up which is required to continue working or to renew health licenses in entertainment establishments. Therefore, the sample size obtained from the clinic in the present study represented the female sex workers throughout the city.

The prevalence of trichomoniasis among 377 women attending the RHWC in Angeles City was 9.55% using PCR primers TV3/7. The prevalence determined in the current study contrasts sharply with the results reported by the sentinel STI etiologic surveillance systems (SSESS) in 2004. This sentinel surveillance system was created to provide regional and national STI programs with current and readily available data on the distribution and frequency of STIs in the country [18]. Groups monitored by SSESS are registered female sex workers (RFSW), freelance sex workers (FLSW), male sex workers (MSW) and injecting drug users (IDUs) in different sentinel STI surveillance sites around the country [19]. SSESS reported no cases of trichomoniasis among these respondents in Angeles City. However, the data on STIs in the Philippines as reported by SSESS are currently limited since STI surveillance has traditionally relied on passive reports from social hygiene clinics (SHCs). Moreover, this is likely to be an underestimate of true prevalence as wet mount microscopy alone was used for the identification of trichomoniasis.

The prevalence in the present study was also higher than that reported by Wi and co-workers in a cross-sectional study in 2002. They detected trichomoniasis in 3.18% of selected female respondents from different parts of the country, and a 3.38% occurrence rate of the parasite among women with symptoms of abnormal vaginal discharge [20]. This study used culture techniques and microscopy to detect *T. vaginalis*.

In direct microscopy or the wet mount method, the most frequently used diagnostic tool for trichomoniasis, the presence of motile trichomonads signifies a positive test. Although direct visualization through microscopy is both rapid and inexpensive, low sensitivity reduces its reliability as a method for *T. vaginalis* detection. The culture method is the current “gold standard” for the diagnosis of trichomoniasis, but it is performed by relatively few laboratories due to the cost of the essential reagents and the laborious nature of techniques such as frequent microscopic observations for positive samples for up to 7 days [21]. The large number of specimens being tested in the present study also limits its use as a detection tool for *T. vaginalis* in vaginal exudates of women. Another potential drawback is that the culture technique may sometimes generate false-negative or false-positive results due to the failure of *T. vaginalis* to thrive in cultures with bacterial and fungal overgrowth over time, as previously observed in our laboratory. It has also been proven that dead *T. vaginalis* cells, which may also be encountered on dried vaginal smears during routine check up, appear dense and granulated and may resemble the nucleus of vaginal epithelial cells.

Alternatively, PCR of vaginal swabs may be advantageous over the latter method of *T. vaginalis* detection especially in settings where incubation of cultures is not possible and shipment of specimens to a reference laboratory is
required [22]. Several groups of investigators have reported findings on the development of a PCR technique for trichomonads. It has been found that the detection of *T. vaginalis* from vaginal swabs by PCR employing primers TV3/7, the primer set utilized in this present study, was equivalent to culture with a sensitivity and specificity of 88.7% and 97.1%, respectively [22]. Also, PCR-based detection of *T. vaginalis* using vaginal specimens may provide an alternative to culture. This is consistent with the findings of Queza (2008) showing that the sensitivity of PCR using primers TV3/7 was comparable to that of culture at 96.97% [12].

One strategy for increasing the diagnosis and treatment of trichomoniasis is the use of a screening test with higher sensitivity compared to the traditional wet prep of vaginal fluid commonly used in social hygiene clinics around the country. In the present study, PCR using primers TV3/7 appears to be highly sensitive, being able to amplify DNA from one *T. vaginalis* cell. This result is consistent with that of Lawing and co-workers who determined that the lower limit sensitivity of PCR with primers TV3/7 was one organism [22]. Therefore, PCR using vaginal swabs and urine specimens, compared to diagnosis by wet mount microscopy, culture, and fluorescent staining, appeared to be the method of choice for the detection of genital infections with *T. vaginalis* [23].

In this study, DNA extractions from samples were performed in batches using the Chelex method. As a chelating resin, Chelex 100 has been used to extract heavy metals from solutions and, when used for DNA extraction, may also eliminate PCR inhibitors present in the samples and/or prevent the disruption of the genomic DNA [14]. The absence of PCR inhibitors and DNA contaminants is essential to the performance of PCR in detecting *T. vaginalis* in vaginal swabs.

Angeles City is a priority area for STI control and HIV prevention. The city has the highest cumulative number of HIV seropositive individuals among the ten HIV surveillance sites in the country [20]. Trichomoniasis has been shown to be a risk factor for transmission and infection with HIV. *T. vaginalis* disruption of urogenital epithelial monolayers could facilitate passage of HIV-1 to underlying cells, and activation of local immune cells by *T. vaginalis* in the presence of infectious HIV-1 might lead to increased viral replication [24]. Therefore, to effectively direct HIV prevention and control programs, a test for *T. vaginalis* infection should be included in routine clinical check-ups among high-risk individuals, just as adequate information on the prevalence and distribution of trichomoniasis should be made readily available. PCR has allowed a greater understanding of the global epidemiology of *T. vaginalis* and raised concerns about the potential impact on HIV transmission and the reproductive health of females [17]. Moreover, significant reductions in the prevalence of a nuisance STI such as trichomoniasis are possible when effective interventions reach core groups such as female sex workers. The correlation of age of female respondents and known risk factors of trichomoniasis is also an important topic for further investigation.

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