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Diagnosis of *Trichomonas vaginalis* using real-time polymerase chain reaction (PCR) among women at Institut Pasteur of Côte d'Ivoire

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Trichomoniasis is a sexually transmitted disease caused by a mobile flagellate pathogenic protist protozoan of the urogenital tract, *Trichomonas vaginalis*. No data is available in Côte d'Ivoire about its diagnosis by molecular methods. This study aims to identify *T. vaginalis* by polymerase chain reaction (PCR) among women at Institut Pasteur of Côte d'Ivoire. Vaginal swabs were obtained from each woman from July to October, 2013. For *T. vaginalis* detection, Giemsa stain for microscopic examination and real-time PCR were performed. The PCR targeted 67 bp region of a repeated sequence of the *T. vaginalis* genome. A positive *T. vaginalis* result was defined as a cycle threshold (Ct) less than 36. The results show that of the 194 specimens tested by both Giemsa stain method and Real-time PCR, 2 were positive to Giemsa stain (1.03%) and 7 were positive in PCR assay (3.61%). Women who had multiple sexual partners in the last two months were found to be the most infected. Comparing the two methods, real-time PCR had a higher sensitivity (100%) and specificity (97.40%). This study shows that the prevalence rate of *T. vaginalis* infection remains low. However, using the PCR approach allows for better detecting infection than conventional staining method.

Key words: *Trichomonas vaginalis*, real-time polymerase chain reaction (PCR), Giemsa stain, Côte d'Ivoire.

INTRODUCTION

Trichomoniasis is a sexually transmitted disease caused by a mobile flagellate pathogenic protist protozoan of the urogenital tract, *Trichomonas vaginalis* (Workowski et al., 2015). This infection is one of the most common non-viral sexually transmitted diseases such as chlamydia, gonorrhea, and syphilis (WHO, 2015). Infection by *T. vaginalis* is considered reportable in most countries and often continues to be considerably high particularly among sexually active young people of reproductive age (Da Ros and Schmitt, 2008). Then, about 70% of women and men do not have symptoms when infected or women have vaginal discharge, smelly, with vulvar irritation, which can be confused with bacterial vaginosis (Schwebke et al., 2011). Some complications can appear

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in untreated women such as endometritis, infertility and cervical erosion. In pregnant women, the infection can cause premature rupture of membranes, preterm deliveries and low-birth-weight infants (Schwebke et al., 2011; Sobel et al., 2013). However, little information about its prevalence is available because of the limited range of diagnosis methods, the lack of screening programs and the absence of notification of this disease (WHO, 2015). Worldwide, the prevalence rates of *T. vaginalis* in women population varies from 5 to 74% according to the methods of diagnosis (Johnston and Mabey, 2008; Korycińska et al., 2017). In United States, trichomoniasis leads to several cases of vaginitis (Brown, 2004); and many women (about five millions) are infected each year (Sobel et al., 2013; Swygard et al., 2004). In Côte d'Ivoire, some studies have reported various prevalences using microscopic observation. Otherwise, studies with related prevalences of 6.9% in 2006 (Abauleth et al., 2006) and more recently 4.5% (Konaté et al., 2014) in Abidjan. Currently, the diagnosis of *T. vaginalis* infection is based on Giemsa stain and culture using genital excretion (Nye et al., 2009; Konaté et al., 2014). Recently, molecular diagnosis by PCR, highly sensitive (80-90%) has been used to detect this parasite, but it is costlier than microscopy and culture (Ginocchio et al., 2012; Schwebke et al., 2018). No data about molecular diagnostic of *T. vaginalis* infection is available in Côte d'Ivoire; hence the aim of this study is to identify *T. vaginalis* by PCR and associated factors among women at Pasteur Institute of Côte d'Ivoire.

MATERIALS AND METHODS

Study design and population

This cross-sectional study was carried out at the Genital Tract Unit (UA-TG) of the Department of Bacteriology Virology of the Pasteur Institute of Côte d'Ivoire, from July to October, 2013. The study population included women with symptoms of sexual transmitted infection, who came for vaginal swabs collection and gave their written consent, in Pasteur Institute of Côte d'Ivoire located in Abidjan (N 5°20′38,112″ W 3°59′44,736″) as a reference laboratory for various clinical samples examination. In this study, women who were not under antibiotic therapy were included, with sexual abstinence for three days and without practicing any personal genital hygiene on the day of collection.

Data collection and Giemsa stain

Women who presented at least one of the symptoms such as vaginal discharge, itching, dysuria, pain in the lower abdomen and provided informed consent were enrolled in this study. An anonymous questionnaire was used and information about socio-demographic data (age, educational level, marital status), sexual contacts (anal sex, condom use, number of sexual partner), type of contraceptive, and history of genitourinary infections were obtained from each woman in private, using structured questionnaires applied by the same investigator. Women with symptoms of sexual transmitted infection were included. Exclusion criteria were patient refusal and inability to give informed consent. Full gynecological examinations were conducted as well. Two vaginal swabs were taken from each patient. The first swab was used to prepare direct examination and microscope slides, which were subject to standard Giemsa stain. The stained specimens were analyzed using a microscope with 1000x power magnification in order to detect pear-shaped trophozoites. The second vaginal swab was put in suspension in phosphate buffer saline (PBS) and kept at -80°C for molecular tests.

Molecular analysis

DNA extraction

The Kit DNA-sorb-AM d'Amplisens was used according to the manufacturer's protocol. Briefly, onto 100 µL of each sample, 300 µL of lysing buffer (chlorate of guanidine) in 1.5 mL microcentrifuge tube was incubated by vortexing at 65°C for 5 min and then at room temperature for 2 min. Tubes were centrifuged at 10000 rpm for 1 min and the supernatant was transferred into a new tube. 1 mL of washing solution was added and incubated at 65°C for 10 min. The eluted DNA was stored at 4°C PCR reaction.

PCR amplification

The target for the real-time PCR assay was a 67 bp region of a repeated sequence of the *T. vaginalis* genome. The primers and probes were designed and used previously by Caliendo (2005). The PCR reaction contained 25 µL of 2X TaqMan Universal Master Mix, 5 µL each (900 nM) of primer TV forward (5'-CATTGACCACACGGACAAAAAG-3') and primer TV reverse (5'-CGAAGTGCTATGCGACGA-3'), 5 µL of probe (225 nM) (5'FAM-TCATTTCGG ATG GTCCAGAAGCCA - TAMRA 3') and 5 µL of sample DNA. The samples were amplified using the following thermal cycling conditions: 50°C for 2 min, 95°C for 10 min, followed by 40 cycles at 95°C for 15 s, and at 60°C for 1 min. Each reaction included a positive and negative control and a positive *T. vaginalis* result was defined as a cycle threshold (Ct) less than 36. The limit of detection was 0.07 organisms per reaction.

Statistical analysis

Statistical analyses were performed with STATA version 15.0. Univariate analysis (χ^2 and Fisher's exact test, as appropriate) was used for comparison between groups. Women were stratified into three age groups (<25, 25-40 and >40 years old). Parasitic infections were defined as positive for *T. vaginalis* when vaginal swabs stain or/and PCR was positive. Significant associations between *T. vaginalis* infection and socio-demographic and habits variables were tested. For all statistical analyses, a p-value below 0.05 was considered significant.

Ethical considerations

The study was conducted within the ethical standards and approved by the National Ethics and Research Committee of Côte d'Ivoire. Informed consent was obtained by participant prior their inclusion in the study.

RESULTS

Population characteristics and sexual habits

A total of 194 women were included in the study. The

Table 1. Prevalence rate of *T. vaginalis* according socio-demographic factors and sexual habits of women.

Variable	Total n (%)	Real-time PCR positive n (%)	p-value
Age (years)			***0.635***
<25	41 (21.13)	2	
25- 40	134 (69.07)	5	
>40	19 (9.79)	0	
Marital status			***0.154***
Single/free union	116 (59.79)	6	
Married	78 (40.21)	1	
Educational level			***0.361***
None	11 (5.67)	1	
Primary	24 (12.37)	2	
Secondary	61 (31.44)	2	
University	98 (50.52)	2	
Anal sex			***0.119***
No	111 (57.22)	2	
Yes	83 (42.78)	5	
Condom use			***0.115***
No	110 (56.70)	6	
Yes	84 (43.30)	1	
Multiple sexual partner last 2 months			***<0.001***
No	184 (95.85)	4	
Yes	10 (5.15)	3	

highest group were women aged 25 to -40 (69.07%), followed by those aged 11 to 12 (21.13%) and more than 40 years (9.79%). The mean age of participants was 31.38 years (standard deviation = 8.54 years) with a minimum of 11 years and a maximum of 73 years. Women with high educational level like university were the most important (50.52%). During sexual contacts, women always or sometimes used condoms (43.30%) and practiced anal sex (42.78%). Women with multiple sexual partners, the last two months (5.15%) were infected by *T. vaginalis* ($p<0.001$). Table 1 shows characteristics of the study population and associated factors of infection.

Symptoms

During the pelvic exams, women reported symptoms such as vaginal itching (46.39%), vaginal discharge (31.96%), dyspareunia (26.80%), pelvic pain (14.95%) and micturition pain (2.58%).

Vaginal swabs stain and real-time PCR

All vaginal swabs were tested microscopically by smear stained and real-time PCR. The prevalence rate of *T. vaginalis* was 1.03% (95% CI: 0.17 - 3.36) by stain method and 3.61% (95% CI: 1.59 - 7.0) by real-time PCR. The real-time PCR assay had a limit of detection of 0.07 organisms per reaction, which was equivalent to approximately 40 copies/ml of specimen (Figure 1). Regarding socio-demographic parameters, *T. vaginalis* was most prevalent in age groups 20 to 40 years old (69.07%), in single women (59.79%), and in women with university level (50.52%), but the difference was not statically significant. Regarding sexual contacts, women who had multiple sexual partners in the last 2 months were the most vulnerable to be infected by *T. vaginalis* ($p<0.001$). Table 1 shows the relationship between the prevalence rate of *T. vaginalis* by socio-demographic factors and sexual habits of women using real-time PCR. Comparing both stain method and real-time PCR, the difference was found to be statistically significant ($p<0.001$). Using stain method as the gold standard, the real-time PCR assay had a sensitivity of 100% and a specificity of 97.40% (Table 2).

DISCUSSION

Studies about prevalence of *T. vaginalis* in Côte d'Ivoire

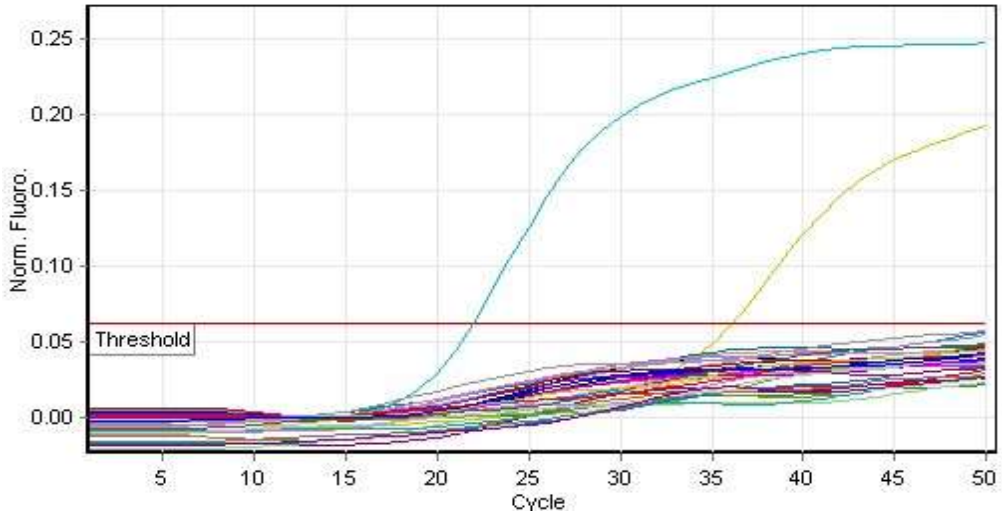

Figure 1. Fluorescence chart produced in real-time PCR.

Table 2. Comparison of swabs stain and Real-time PCR for the detection of *T. vaginalis* in vaginal swabs.

Real-time PCR	Microscope examination		
	Positive	**Negative**	**Total**
Positive	2	5	7
Negative	0	187	187
Total	2	192	194

Real-time PCR: Sensitivity= 100%, Specificity= 97.40%, p-value<0.05.

use usually insensitive methods, like microscopic examination of vaginal swabs stained (Abauleth et al., 2006; Konaté et al., 2014). In addition, *T. vaginalis* infections are not reportable to public health officials and are generally not part of routine screening and in many cases the infection is asymptomatic. The prevalence rate of this infection is underestimated because of the low sensitivity of Giemsa stain method. The current study is the first to use a sensitive method such as real-time PCR to determine *T. vaginalis* prevalence in women population in Côte d'Ivoire, and compare it to swabs stain method. The low prevalence reported in this study was also found in European countries (Field et al., 2018; Hilmarsdottir et al., 2017; Trevisan et al., 2008). In a population, assessment of prevalence rate of *T. vaginalis* depends on the choice of diagnostic methods. Each method has its requirements which depend on the test material, the study population, the laboratory tools and financial support (Korycińska et al., 2017).

The prevalence of *T. vaginalis* was found to be higher using PCR in the present study with sensitivity of 100% and specificity of 97.40% comparing to Giemsa stain method. This study confirms some others results (Caliendo et al., 2005; Cwikel et al., 2008; Joura et al., 2015; Muñoz-Ramírez et al., 2018) showing that PCR testing was more sensitive than stained swabs and culture for the detection of *T. vaginalis*. However, data found in the literature suggested a high sensitivity of PCR ranging from 82 to 95% and specificity of 97 to 100%, as compared to the microscopic test (Korycińska et al., 2017). In this study, the real-time PCR assay detected about 70% more positive samples than Giemsa stain method.

Socio-demographic characteristics reported in the present study showed that sexually active women were found in ages 15 to 40, some of them being minors, with low schooling level, single or in free union; this is similar to results from other studies (Leyva-Flores et al., 2013; Semple et al., 2015). These findings are contradictory with those that found that *T. vaginalis* infection affected women of all ages and its prevalence was the highest in women aged over 40 years and lowest in women under 40 years (Andrea and Chapin, 2011; Freeman et al., 2010; Ginocchio et al., 2012; Sutton et al., 2007). However, it may be seen that the highest number of cases of trichomoniasis occurred in the group of women

aged 50 to 59 and those over 60 years (Korycińska et al., 2017). Among women presenting with symptoms, vaginal discharge was the most common etiology of *T. vaginalis* infection. This has been reported recently in a study about sexual transmitted infections (Chirenje et al., 2018).

In the present study, women who had a greater number of sexual partners in the last two months were the most infected. These findings are similar to those reported in other studies which showed a great link between having multiple sexual partners and the occurrence of *T. vaginalis* infection (Davis et al., 2018).

This study had some limitations. Population characteristics such as gender (only women) and numbers of sexual partners were not assessed. The study included a few sample among women.

In conclusion, this study shows that the prevalence rate of *T. vaginalis* infection remains low using Giemsa stain for microscopic examination.

However, using the PCR approach allows for better infection detection than conventional staining method.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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