PREVALENCE OF *TRICHOMONAS VAGINALIS* IN RELATION TO SEXUAL BEHAVIOUR AMONG FEMALE SEX WORKERS VISITING SEX WORKERS OUTREACH PROGRAM CLINICS IN NAIROBI, KENYA

BY

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156/82221/2012

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTERS OF SCIENCE IN APPLIED PARASITOLOGY

SCHOOL OF BIOLOGICAL SCIENCES

UNIVERSITY OF NAIROBI

SEPTEMBER 2016

DECLARATION

I hereby declare that this is my original work and to the best of my knowledge, has not been submitted to any other university for examination.

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DEDICATION

I dedicate this work to the female sex workers who participated in this study without whom the study could not be a success.

ACKNOWLEDGEMENT

I take this opportunity to express my sincere gratitude to my supervisors Dr. David Odongo, Dr. Julius Oyugi, and Dr. Joshua Kimani, for allowing me to carry out my study at SWOP clinics and for their valuable guidance, constant supervision and dedication and making my research work successful.

I would like to express my special gratitude and thanks to Dr. Moses Masika at the Dept of Medical Microbiology, University of Nairobi for his help in data analysis.

I wish to express my sincere thank you to SWOP clinics staff for their support and help in collecting and transporting of the study samples.

My thanks and appreciation also goes to the UNITID staff (Irungu, Wendy, Nyakio, Njeri) and also to my colleague Mr. Mbithi in Medical Microbiology for their encouragement and support during the course of this study.

I would also like to specially thank my son Ernest Muthini, my niece Stacy Nziva and my sister Patra Mutanu for their motivation, love, spiritual and moral support throughout my research period.

Thank you all and may the almighty God bless you abundantly.

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LIST OF ABBREVIATIONS

ARVS	Anti retrovirals
BSA	Bistrimethylsily acetamide
BTUB	Beta tubulin
DNA	Deoxyribonucleic acid
DATP-	Deoxyadenosine triphosphate
DCTP	Deoxycytidine triphosphate
DGTP	Deoxyguanosine triphosphate
DTTP	Deoxythymidine triphosphate
DNTP	Deoxyribonucleotide Triphosphate
DUTP	Deoxyuridine-5-Triphosphate
FSW	Female Sex Workers
HIV	Human Immunodeficiency Virus
HVS	High vaginal Swab
IDU	Injection Drug Users
KAVI	Kenya Aids Vaccine Initiative
MARPS	Most at Risk Populations
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
SPSS	Statistical Package for Social Science
STD	Sexually Transmitted Diseases
STI	Sexually Transmitted Infections
SWOP	Sex Workers Outreach Program
UNG	Uracil N Glycosidase
UNITID	University of Nairobi Institute of Tropical and Infectious Diseases
UOM	University of Manitoba
UON	University of Nairobi

ABSTRACT

Sexually transmitted infections (STIs) are a major public health problem, especially in developing countries. *Trichomonas vaginalis* is a sexually transmitted pathogenic protozoan parasite of worldwide importance and causes a disease known as Trichomoniasis. The infection causes adverse reproductive health and pregnancy outcomes and has also been recognized to play a critical role in HIV acquisition and transmission. Commercial sex work is a risk factor likely to expose persons to infection with *T. vaginalis* and it is therefore of interest to screen for *T. vaginalis* among female sex workers. The prevalence of Trichomoniasis among sex workers in Kenya has not been extensively studied and there is lack of guidelines for screening of sex workers. Knowledge of the prevalence of curable Trichomoniasis among this population would provide basis for integrating *T. vaginalis* screening in SWOP clinics. This study determined the prevalence of *T. vaginalis* infection among female sex workers attending SWOP clinics within Nairobi County. Following informed consent, wet mount microscopy and PCR were used to test for presence of *T. vaginalis* in vaginal swab samples obtained from 150 female sex workers who visited 3 SWOP clinics in Nairobi, and who presented with vaginal discharge. A questionnaire on socio-demographic, behavioral and gynecological characteristics was also administered. Of the 150 women tested, microscopy detected *T. vaginalis* in 7/150 (4.7%) patients while PCR detected *T. vaginalis* in 19/150 (12.7%) patients. Detection by wet mount microscopy demonstrated a low sensitivity of 31.6% and specificity of 99.2%. All wet mount positive samples were re-examined to detect viability of trichomonads at hourly interval for up to 5 hours. Motility of *T. vaginalis* trophozoites was observed to decrease after every subsequent hour and by the 5^{th} hour all the trophozoites were immotile. Risk of *T. vaginalis* infection among the sex workers was significantly associated with inconsistent condom use with regular clients. The

prevalence of *T.vaginalis* was slightly higher in women who were HIV positive, had multiple sex partners, had many years in sex work, and practiced unprotected sex and vaginal douching. The study showed that, the prevalence of *T.vaginalis* among female sex workers was relatively high and PCR was the most sensitive tool rather than microscopy which has been routinely used to detect *T. vaginalis* infections in Kenya. Therefore from the notable prevalence of *T. vaginalis* infections detected by PCR among female sex workers, clinicians should consider routine diagnosis of Trichomoniasis by this method in high risk individuals in order to prevent persistent infection and transmission of this disease. This study therefore reinforces the need to implement regular screening for Trichomoniasis among sex workers attending SWOP clinics using a highly sensitive technique.

CHAPTER ONE

BACKGROUND AND LITERATURE REVIEW

1.1 BACKGROUND.

Trichomonas vaginalis is one of the most common curable sexually transmitted protozoan parasites in humans and causes a disease known as Trichomoniasis(Kim *et al*., 2016). Nearly more than half of women and men infected with *T.vaginalis* are asymptomatic with approximately 1/3 of asymptomatic women becoming symptomatic within 6 months following infection (Kissinger, 2015a). The classical presentation of *T.vaginalis* infection is that of purulent ,foul smelling vaginal discharge associated with pruritis, dysuria and dyspareunia (Garber, 2005). Among the infected women 2% may present with straw berry cervix characterized by punctuate hemorrhagic lesions (Swygard *et al*., 2004).Untreated Trichomoniasis in women can cause cervicitis, vaginitis and pelvic inflammatory disease (Moodley *et al*., 2002, Marrazzo and Martin, 2007, Jahic *et al*., 2013).

Local inflammatory responses to *T. vaginalis* infection by the host predispose pregnant women to premature rupture of membrane, preterm birth and low birth weight babies (Sutton *et al*., 2007, Coleman *et al*., 2013). *T. vaginalis* has also been reported to contribute to high risk of cervical cancer in women and HIV acquisition and transmission (Zhang *et al*., 1995, McClelland *et al*., 2005).

In men, Trichomoniasis is usually asymptomatic hence males serve as asymptomatic carriers making it important to treat male partners of infected women to avoid re- infection. Occasionally the infection with the parasite in men may cause non-gonococcal urethritis with non purulent urethral discharge (Schwebke and Hook, 2003). Symptoms of Trichomoniasis in males include

clear mucopurulent discharge and dysuria and the infection can cause balanoposthisis, epididymitis, prostitis and infertility in men (Cudmore *et al*., 2004).

Female sex workers have been widely reported to enhance the spread of Trichomoniasis and other sexually transmitted infections to the general population and therefore screening and treatment of *T.vaginalis* in this group can aid in the control of this disease(Shrestha *et al*., 2016). However, little effort has been made to improve the diagnosis of *T.vaginalis* in our local settings and there are no existing guidelines for the screening of this parasite. Currently the commonly used technique in estimation of *T.vaginalis* in SWOP clinics and other Kenyan facilities is wet mount microscopy that detects motile trichomonads in urine, vaginal and urethral fluids by visual examination of the specimens under a microscope. This method has been reported as insensitive compared to molecular DNA amplification techniques and the chances of missing the parasites are high (Ginocchio *et al*., 2012, Soba *et al*., 2015). Furthermore treatment of patients infected with Trichomoniasis in our local settings is based on signs and symptoms regardless of the asymptomatic nature of this disease. Indeed, the prevalence of *T.vaginalis* by wet mount microscopy has been underestimated and the true prevalence is not known.

The purpose of this study was to determine the prevalence of *T.vaginalis* among female sex workers by wet mount microscopy and PCR techniques as well as determining sexual behavioral factors associated with positive *T.vaginalis* and also finding out the viability of *T.vaginalis* trophozoites in collected High Vaginal Swabs (HVS) preserved in normal saline.

1.2.0 LITERATURE REVIEW

1.2.1 Introduction

Trichomonas vaginalis is an anaerobic flagellated protozoan parasite of worldwide importance(Kissinger, 2015a).The infective stages are the flagellated trophozoites which are found within the urogenital tract of both women and men causing a disease known as Trichomoniasis (Cheesbrough, 1987).

1.2.2 Classification of *T. vaginalis*

The parasite was first visualized by Donne in 1836 (Thorburn, 1974) and classified as follows;

Domain- Eukarya

Phylum-Metamonada

Class-Parabasalia

Order-Trichomonadida

Genus-Trichomonas

Species-*Trichomonas vaginalis*

1.2.3 Morphology of *T. vaginalis*

T. vaginalis is ovoid in shape and measures between 7-23µm in length and 5-12µm in width. The parasite has no known cyst stage and exists as a trophozoite. In a wet preparation the trichomonad has a characteristic jerky motility brought about by four anterior flagella embedded in an undulating membrane which runs along two thirds of the cell (G.C.Cook, 1996) as shown in Figure 1 below. *T. vaginalis* is phagocytic and grows in moderately anaerobic conditions.

It reproduces through binary fission and requires carbohydrates as an energy source (Coleman *et al*., 2013).

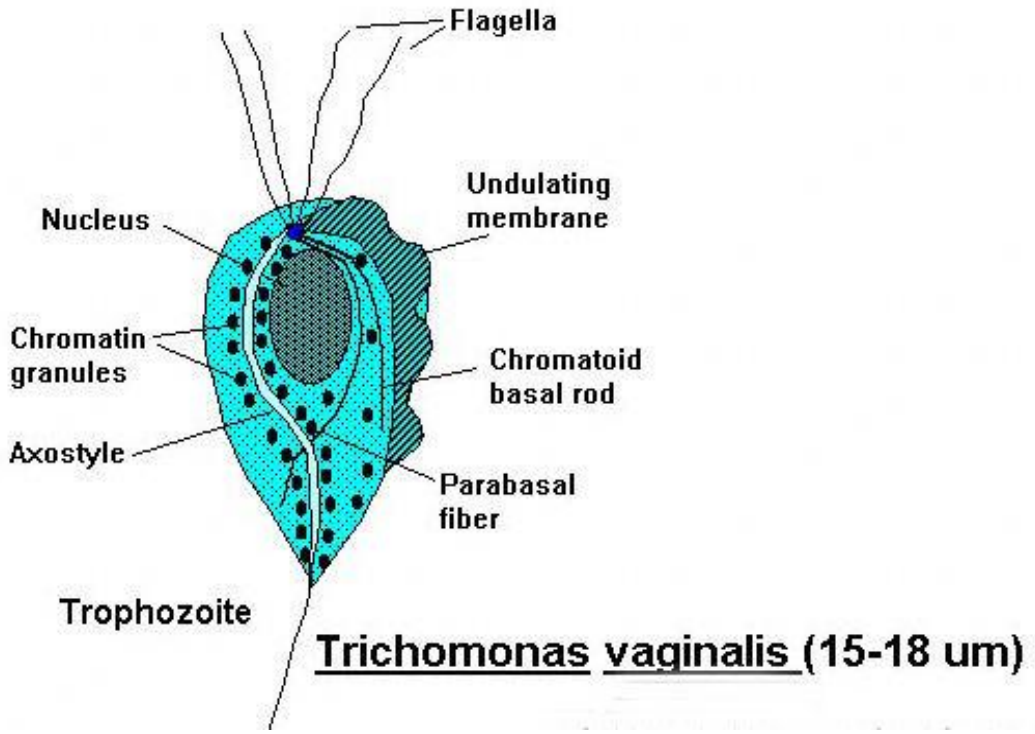

Figure 1: Morphological form of *Trichomonas vaginalis*. (Asm microbel library.orgodel castili)

1.2.4 Mode of transmission of *T. vaginalis*

Sexual transmission predominates and is the most important mode of transmission of Trichomoniasis. Non-sexual transmission is rare but has been observed in cases involving shared bathing implements, inconsistent use of soap and contaminated water (Charles, 1991, Adu-Sarkodie, 1995, Crucitti *et al*., 2011). Perinatal transmission to newborn from infected mothers with *T. vaginalis* is also possible and can result in vaginal and respiratory tract infections (Schwandt *et al*., 2008, Carter and Whithaus, 2008).

1.2.5 Life cycle of *T. vaginalis*

The life cycle of *T. vaginalis* is poorly understood (Petrin *et al*., 1998). As shown in Figure 2 below, the parasite has no known cystic stage. The trophozoite is transmitted through coitus and reproduces by binary fission giving rise to a population in the lower genital tract of females and in the urethra and prostate in males(Schwebke and Burgess, 2004).

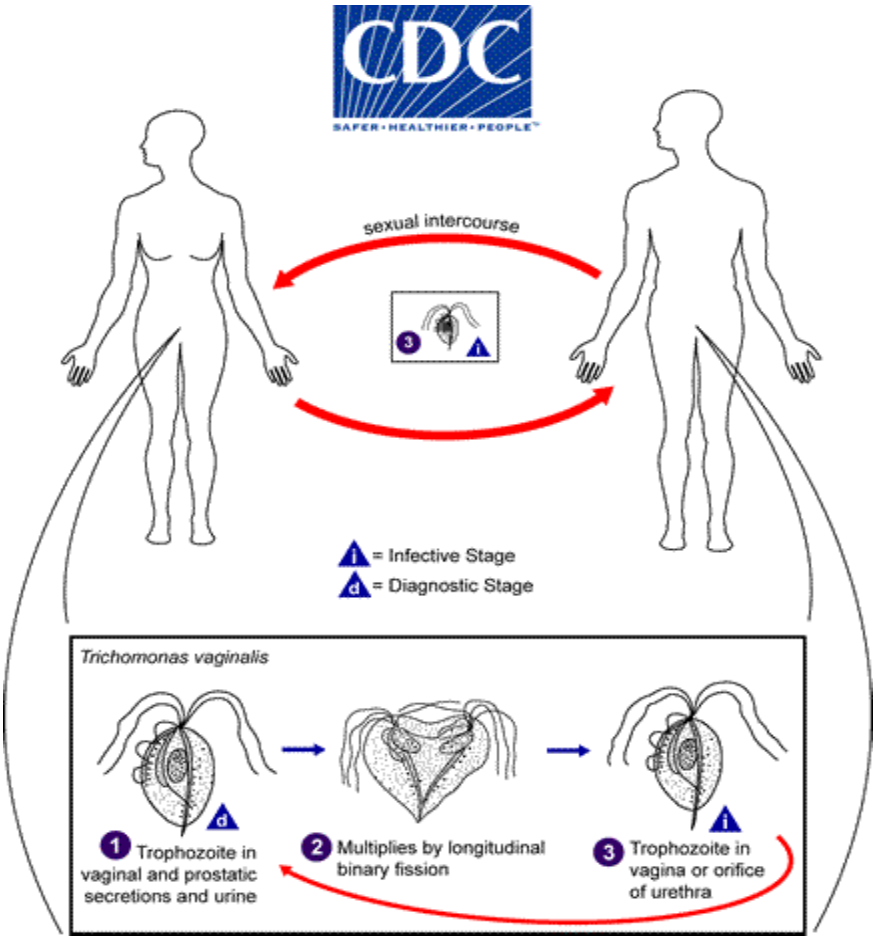

Figure 2: The life cycle of *Trichomonas vaginalis* (http//www.dpd.cdc.govt .pdx)

1.2.6 Prevalence of Trichomoniasis

The World Health Organization 2008, global incidence and prevalence of selected curable sexually transmitted diseases estimated the incidence of *T.vaginalis* as 276.4 million cases per year. Trichomoniasis is cosmopolitantly distributed and has been identified in all racial groups and socio economic strata (Seema sood, 2008).The prevalence of *T.vaginalis* varies with geographical location,diagnostic technique and study setting(Kissinger, 2015b). Earlier studies have reported a higher prevalence of the disease in black women at 13.3% than in white women at 1.3% (Bachmann *et al*., 2011).The disease occurs during the reproductive age and the infection has been found to be commoner in women than in men (Sutton *et al*., 2007). Trichomoniasis is frequently diagnosed in pregnant women with studies in sub-Saharan Africa indicating the prevalence among pregnant women, in Zimbabwe at 13%, Uganda at 15.9% and Kenya between 20 -23% (Feldblum *et al*., 2000, Fonck *et al*., 2000, Mullick *et al*., 2005).

Sex workers represent a high risk group which is capable of transmitting Trichomoniasis and other sexually transmitted infections to the general population. Past studies have reported a high prevalence of *T. vaginalis* in this group by various methods. For example, the prevalence of Trichomoniasis among street-based female sex workers in Bangladesh and in low income female sex workers in Mongolia was estimated at 45.5% and 28% respectively by wet mount microscopy (Rahman *et al*., 2000, Hagan and Dulmaa, 2007). However, in Pakistan low prevalence of 5.1% was reported by (Khan *et al*., 2011). A similar trend has also been shown when *T. vaginalis* infection is detected by other diagnostic methods including culture. For instance in Uganda the prevalence of *T. vaginalis* among a cohort of women involved in high risk sexual behaviour was 17% by culture (Vandepitte *et al*., 2011) while in Indonesia the prevalence of *T. vaginalis* among sex workers in nine provinces was estimated at 15.1% by the

same method (Tanudyaya *et al*., 2010). Various results have been reported when PCR technique is used to detect *T. vaginalis* infection and the prevalence of Trichomoniasis among commercial sex workers ranges from 41% in Papua Guinea,33.2% in Ndola Zambia, 22.6% in Manado Indonesia to 6.8% in Philippines (Bruce *et al*., 2010, Crucitti *et al*., 2010, Mawu *et al*., 2011, Queza and Rivera, 2013) .

In Kenya, there has been a general decline in the prevalence of *T. vaginalis* among female sex workers and this is due to establishment of HIV/STI intervention programs for this group. A study conducted among female sex workers in Kisumu, reported a decline in prevalence of *T. vaginalis* from 45.2% in 1997 to 13.6% in 2008 (Vandenhoudt *et al*., 2013). In another survey conducted in Nairobi on biological and behavioral surveillance for most at risk population including homosexuals, heterosexual female sex workers (FSW) and injecting drug users (IDU), a prevalence of *T. vaginalis* in FSW of 10.3% was reported and zero among homosexuals(MARPS Surveillance Report 2012). Furthermore, a recent survey which was carried out in Kariobangi clinic in Nairobi county which was one of the clinics included in this study reported a prevalence of *T. vaginalis* among female sex workers at 7.2% (Gomih-Alakija *et al*., 2014).

1.2.7 Risk factors associated with Trichomoniasis

Prevalence of Trichomoniasis and other sexually transmitted diseases in women have been associated with many behavioural risk factors. Some studies have associated Trichomoniasis and STI's with symptomatic partner, new sex partner, unfaithful partner, prostitution and joblessness (Behets *et al*., 2005). Other studies have associated Trichomoniasis with older age, multiple sex partners, less education , poverty, being married, unprotected sex and being unemployed (Verteramo *et al*., 2008, Fernando *et al*., 2012, Perla *et al*., 2012). Besides the above mentioned

factors, studies have also associated *T. vaginalis* infections with non-Hispanic black race/ethnicity (Sutton e*t al*., 2007). In contrast with the above studies, multiple sex partners and inconsistent condom use have been reported to have no relationship with incidence of *T. vaginalis* infection while cigarette smoking and alcohol use were found to be associated with Trichomoniasis (Swartzendruber A, 2014). Other behavioral factors such as vaginal douching (a practice of washing or flushing the vagina with water and other fluids) has been reported to play a role in increasing genital tract infections in both HIV infected and uninfected individuals. Past literature has also associated vaginal douching with demographics such as age, education and socioeconomic status, physical , mental health and STI's status among adolescent females (Clark, 2007, Sutton et al., 2007, Hiber AM, 2010, Diclemente *et al*., 2012).

1.2.8 Co-infection of *T. vaginalis* with other sexually transmitted infections

T. vaginalis co-exists with other sexually transmitted infections including *Chlamydia trachomatis*, *Neisseria gonorrhea*, *Herpes simplex* 1 and 2 and syphilis (Allsworth *et al*., 2009, Ginocchio *et al*., 2012). This protozoan parasite predisposes women to bacterial vaginosis characterized by change in vaginal flora (Marx *et al*., 2010, Rathod *et al*., 2011). Co-infection of *T. vaginalis* with *Mycoplasma genitalium* has been reported as a common non gonococcal urethral syndrome through inflammatory response of the host (Coleman *et al*., 2013, Gomih-Alakija *et al*., 2014).

Infection with *T. vaginalis* has been recognized to play a critical role in HIV acquisition and transmission and may have a major impact on the HIV epidemic (Sorvillo *et al*., 2001). The proposed mechanisms on how *T. vaginalis* amplifies HIV transmission are the following; *T. vaginalis* infection elicits an aggressive local cellular immune response with inflammation of the vaginal epithelium and exocervix in women and the urethra of men. This inflammatory

response induces the recruitment of leucocytes including HIV target cells such as $CD4^+$ lymphocytes and macrophages to which HIV bind and gain access. *T. vaginalis* also causes punctuate mucosal hemorrhages which potentially compromise the mechanical barrier to infections (Kissinger, 2015a).

Data from a 10 year prospective study of sex workers in Mombasa Kenya has shown association between *T. vaginalis* and HIV infection by 1.52 fold (McClelland *et al*., 2005).Similarly, a nested case control study which was carried out in Uganda and Zimbabwe strongly confirms this out of 213 seroconverters 24(11.3%) were diagnosed with *T. vaginalis* prior to HIV-1 sero-conversion (Van Der Pol *et al*., 2008).

1.3 Diagnosis of *T. vaginalis*

1.3.1 Diagnosis of *T. vaginalis* by direct wet mount microscopy

The traditional and most commonly used technique in Kenya for diagnosis of *T. vaginalis* infection is light microscopic examination of directly mounted wet smears of vaginal or urethral discharge. Immediate microscopic examination of smears for live trophozoites is usually inadequate for detection of infections (Soba *et al*., 2015). However, although this technique is inexpensive and rapid, it has a few disadvantages requiring a trained microscopist. In addition, its detection threshold and sensitivity is low at about 60%-70%, and can decrease to 20% if microscopic examination is delayed by 10 minutes (Bachmann *et al*., 2011). Furthermore, microscopy does not frequently detect infections in which the number of parasites is low resulting in high false negative diagnosis. Possible delays in examination due to transportation of specimen from the clinic to a distant laboratory may result in death of the trophozoites leading to

false negative results. It may also be difficult to microscopically differentiate *T. vaginalis* from intestinal *Trichomonas hominis,* which may contaminate the specimen during sample collection.

1.3.2 Diagnosis of *T. vaginalis* by culture technique

Detection of *T. vaginalis in vitro* in Diamond's medium and inpouch TV culture has been used as a gold standard for diagnosis of *T. vaginalis* and has proved to be more sensitive than wet mount microscopy. The sensitivity of culture detection method ranges between 44%-95 % (Soba *et al*., 2015). For the trophozoites to be viable and be detected by culture, collected HVS/urethral swabs require immediate inoculation into the TV inpouch culture medium and incubated at 37°C. A major limitation of the culture technique is that the turnaround time is long and may take 5-7 days with daily examination for motility of trichomonads. In addition, culture cannot be carried out in places without thermal incubators and the culture media is also expensive (Lazenby, 2011).

1.3.3 Molecular detection of *T. vaginalis* by Polymerase Chain Reaction (PCR)

For early and accurate diagnosis of Trichomoniasis in both symptomatic and asymptomatic cases, highly sensitive, accurate and rapid techniques are required. Currently, molecular diagnostic kits and techniques which amplify target *T.vaginalis* nucleic acid sequences are available (Madico *et al*., 1998, Caliendo *et al*., 2005). These techniques include the Aptima™ *T. vaginalis* Nucleic Acid Amplification Assay (manufactured by Gene probe) which has a detection sensitivity of 74-98% and specificity of 87-98% and can be used to detect *T. vaginalis* rRNA in various specimens including urine, endocervical and vaginal swabs (Ginocchio *et al*., 2012). Several PCR tests for detecting *T. vaginalis* DNA have been described and compared to wet mount and culture technique and shown to be more sensitive. For instance, a study by (Wendel *et al*., 2002) reported the sensitivity of wet mount at 52%, culture 78% and PCR 84%.

Similarly other past reports have reported a higher sensitivity of PCR at 97% and specificity of 98% (Madico *et al*., 1998) compared to low sensitivity of wet mount microscopy reported to range between 38% and 60% respectively (Patil *et al*., 2012, Nathan *et al*., 2015). Other than the higher sensitivity of PCR techniques, molecular techniques have the advantages of detecting both live and dead trichomonads and are not limited by immediate transportation and rapid processing of sample and can detect low numbers of Trichomonads in a specimen.

1.4 Motility of *T. vaginalis* in collected High Vaginal Swab (HVS).

Little information is available on longevity of *T. vaginalis* in collected vaginal discharge stored at room temperature. *T. vaginalis* may be under diagnosed in wet mount microscopy as a result of delay in examination of sample or evaporation of moisture from the specimen reducing motility of these parasites .A study by (Kingston *et al.*, 2003) reported that, out of 65 positive specimens with *T. vaginalis*, 13 (20%) became negative after 10minutes, 23 (35%) by 30 minutes, 51 (78%) by 2 hours with the few left exceeding 2 hours. Other previous studies have suggested that by preserving the swabs in normal saline after collection, motility of the Trichomonads was 100% within 30 minutes, 99% in 60minutes and decreases to between 3-15% after each subsequent hour (Stoner *et al*., 2013).

1.5 Treatment of Trichomoniasis

Trichomoniasis can be cured with a single dose of 2g Metronidazole (also known as Flagyl®) or 2g Tinidazole given orally, though resistance to these drugs has been reported (Schwebke and Barrientes, 2006). Currently, repeat infections as high as 36% have been found in HIV positive women. This has been attributed to treatment failure and re-infections from multiple sexual partners (Conrad *et al*., 2013).

1.6 Prevention and control of Trichomoniasis

Preventive measures include; education and counseling of persons at risk on ways to avoid sexually transmitted diseases through changes in sexual behaviors and use of recommended prevention services e.g. condoms. Effective diagnosis, treatment, and follow up of infected persons are also important. Sex partners of persons who are infected with any sexually transmitted diseases should be counseled and treated appropriately (CDC, 2015).

1.7 Justification and significance of the study

T. vaginalis is the leading curable sexually transmitted infections in the world (Kissinger, 2015b). This parasite has been associated with pelvic inflammatory disease, cervicitis, vaginitis, preterm birth in pregnant women while in men it has been linked with non gonococcal urethritis. In both sexes Trichomoniasis has been recognized to play a critical role in HIV acquisition and transmission. An active surveillance, accurate diagnosis and effective treatment of the high risk population diagnosed with *T. vaginalis* could lead to reduction of Trichomoniasis and the linked clinical sequelae.

Prevalence of *T .vaginalis* among female sex workers in Kenya is at 10.3% as per 2012 MARPS Surveillance Report, there is need to screen sex workers from time to time in an effort to control and eliminate this neglected disease. The MARPS report was based on PCR technique which is expensive and not routinely carried out in our local laboratory settings, hence, it is of interest to compare the performance of routine technique wet mount microscopy to that of PCR for the detection of *T. vaginalis* in addition to finding out optimal timeline between collection and microscopy of samples for accurate diagnosis of *T. vaginalis* by wet mount microscopy.

1.8 RESEARCH QUESTIONS

1) What is the prevalence of *T.vaginalis* among female sex workers attending Sex Workers Outreach Program clinics in Nairobi?
2) What are the behavioral risk factors associated with *T.vaginalis* infection?
3) How long can *T.vaginalis* trophozoites survive in normal saline after sample collection?
4) What is the sensitivity and specificity of wet mount microscopy compared to PCR techniques?

1.9. Objectives

1.9.1 General Objective

To determine the prevalence and reproductive health factors associated with *T. vaginalis* infection in female sex workers and to evaluate how time taken before wet mount microscopy affects the sensitivity of the test.

1.9.1.2 Specific Objective

The specific objectives of the study were;

1) To assess the prevalence of *T. vaginalis* among female sex workers attending Sex Workers Outreach Program (SWOP) clinics.
2) To determine sexual behavioral habits associated with acquisition of *T. vaginalis* infection.
3) To determine effects of time on viability and detection of *T. vaginalis* infection.
4) To evaluate the performance of wet mount microscopy and PCR methods in detecting *T. vaginalis*.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Study area

The study was carried out at Sex Workers Outreach Program (SWOP) clinics in Nairobi County. SWOP is a leading sex workers agency in Kenya that promotes the health safety and well being of female sex workers, homosexuals and injecting drug users. All sex workers who attend these clinics are offered free Voluntary Counseling and Testing (VCT) for HIV, health education on other STI's, free condoms and free access to general health care. Currently, 9 SWOP clinics are operational within Nairobi County. Three SWOP clinics namely; Kawangware, Thika Road and Kariobangi SWOP clinics were randomly selected for this study.

2.1.2 Description of the study clinics

a) **Kariobangi SWOP clinic**: This clinic is located in Korogocho on the North eastern side of Nairobi city and it is among the oldest SWOP clinics. Neighboring this area are also informal slums settlements (Huruma, Mathare, Dandora and Baba Ndogo) whose residents benefit from the clinic. More than 5,000 sex workers are enrolled in the clinic and at least 20 are served at the facility per day.

b) **Kawangware SWOP clinic: -** This clinic is located in Kawangware which is a moderately populated slum area on the western side of Nairobi city. More than 2400 sex workers are enrolled at the site and approximately 15 of them seek health care services per day.

c) **SWOP Thika road clinic:** This clinic is located in Roysambu area along Thika Road in Kasarani district, Nairobi County. This clinic is not located in slum area but it serves sex workers from Kasarani area (middle class estate) and neighbouring Baba Ndogo slums. More than 4000 sex workers are enrolled in this clinic and each day the clinic receives more than 20 sex workers.

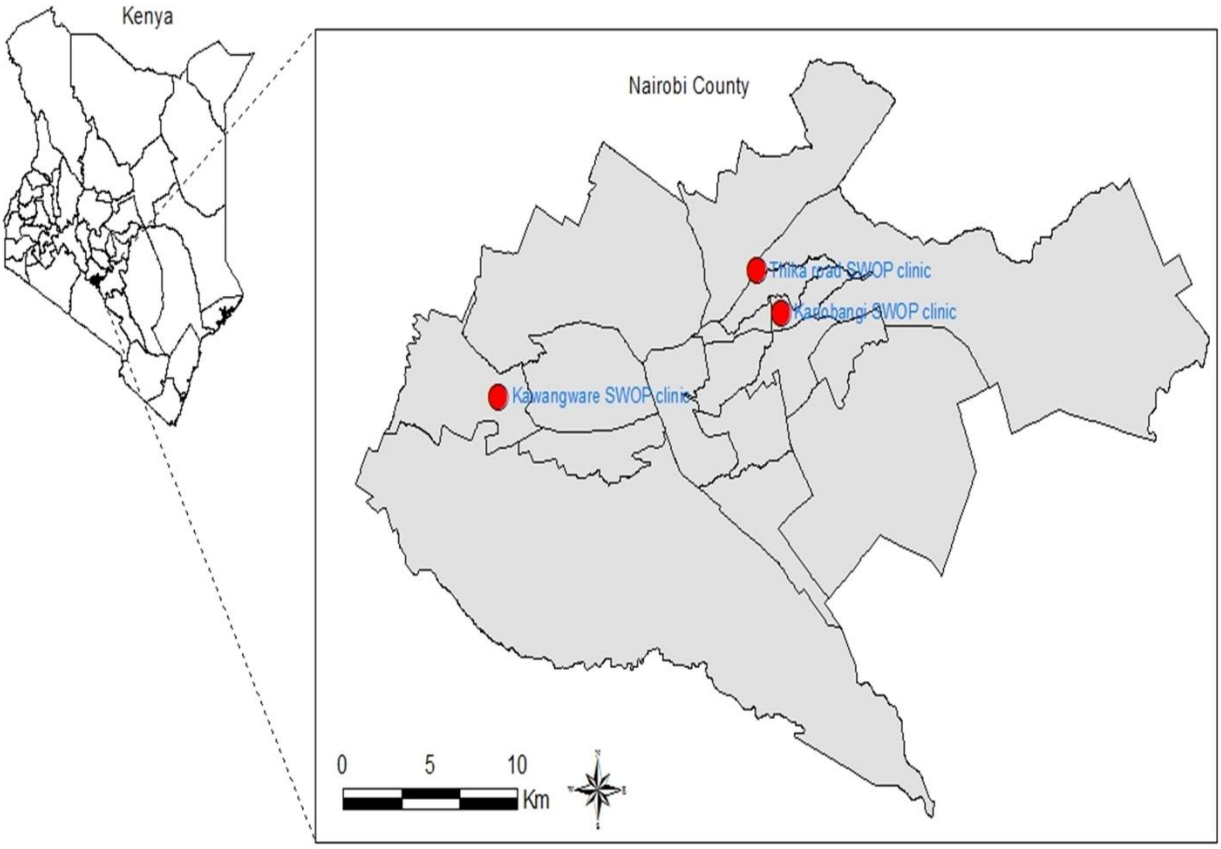

Figure 3: Map of Kenya highlighting Nairobi County and the location of the SWOP clinics where the study was conducted.

2.2 Study design.

A cross-sectional study was conducted between September and December 2014 and all eligible women who attended the SWOP clinics during the study period were sampled.

2.3 Inclusion and Exclusion Criteria

2.3.1 Inclusion Criteria

The female sex workers who were included in this study met the following criteria;

a) Women who signed informed consent to participate in the study

b) Women who were aged 18 years and above

c) Women who attended the clinic and presented with vaginal discharge

d) Women not on any treatment for vaginal discharge and who had not been treated for vaginal discharge in the last 30 days.

2.3.2 Exclusion Criteria

a) Women who did not sign informed consent to participate in the study

b) Women who were aged below 18 years

c) Women who did not present with vaginal discharge

d) Women who were on treatment for vaginal discharge in the last 30 days.

2.4 Sample size calculation

The Jaykaran and Tamoghna, (2013) formula for calculating sample size was used in this study. Available data from (MARPS Surveillance Report, 2012) in Kenya reported the prevalence of *T. vaginalis* at 10.3%. Assuming that 10.3% of those enrolled for the study were diagnosed with *T. vaginalis*, a 95% confidence interval and limiting the absolute error to 5% then;-

$$n = \frac{Z^2_{1-\alpha} x P (1-P)}{d^2}$$

$$n = \frac{1.96^2 x 0.103 x 0.897}{0.05^2}$$

n=minimum sample size

z=is normal deviate corresponding to alpha=1.96

p=is the estimated prevalence=10.3

d=is margin of error/absolute precision=0.05

The minimum sample size based on this calculation was 142 participants.

This study recruited 150 participants from the 3 clinics representing 50 sex workers from each SWOP clinic. The additional 8 participants were recruited to take care of drop outs.

2.5 Specimen collection and processing

2.5.1 Collection of high vaginal swab.

Pretest counseling on sexually transmitted infections was provided to all patients who visited the SWOP clinics by a trained nurse. Women who were willing to participate in the study signed an informed consent (**Appendix 1**) and were taken through a questionnaire on demographic,

behavioral and obstetrical characteristics (**Appendix 2**).(The consent form and the questionnaire were translated into Kiswahili(National language) or any other language the participants could understand by the study nurse) .Following this, two high vaginal swabs from each subject were obtained from the posterior fornix of the vagina by gently rolling sterile swab 2-3 times with the aid of plastic disposable speculum. The swabs were then labeled appropriately with the client study number only, for the purpose of confidentiality. One swab was immersed in plastic tube containing 1 ml normal saline (0.85% sodium chloride solution) while the second high vaginal swab was packed and stored in a cool box then transported to the Department of Medical Microbiology University of Nairobi and stored at -20^{o}C until further processing. The study participants were not tested for HIV infection during this study. Data on HIV status of each participant was obtained from SWOP clinics records.

2.5.2 Detection of *T. vaginalis* by direct wet mount microscopy

The first swab was emulsified in two drops of normal saline on a microscope slide and a cover slip applied. The slide was examined under Olympus microscope with x10 and x40 objectives within 30 minutes of sample collection. Any positive specimen was re-examined after 1, 2, 3, 4 and 5 hours intervals. All the swabs which were examined at the clinic were also transported to University of Nairobi Institute of Tropical and Infectious Diseases (UNITID) laboratory and re-examined by a second independent microscopist. All patients who tested positive for *T. vaginalis* as determined by wet mount microscopy were treated on the same day at the clinic with 2g Metronidazole.

2.5.3 Detection of *T. vaginalis* by polymerase chain reaction (PCR)

2.5.3.1 Extraction of *T. vaginalis* genomic DNA

DNA was extracted from the second high vaginal swabs collected from participants using the QIAmp® DNeasy blood and tissue kit (Qiagen-Germany) according to the manufacturer's instructions. Briefly, the swabs were re-suspended in 200 µl of Phosphate buffered saline (PBS) and mixed with 20 µl of proteinase-K and 200 µl of AL lysing buffer. The lysate was vortexed for 15 seconds and incubated in water bath at 56°C for 10 minutes. The tubes were left to cool and then centrifuged briefly. Absolute ethanol (200 µl) was added to the sample, mixed thoroughly by vortexing for 15 seconds.

The mixture was then transferred into DNeasy mini spin columns placed into a 2 ml collection tubes and centrifuged at 8000 rpm for 1 minute and the flow through discarded together with the collection tubes. The DNeasy mini spin columns were placed into a new 2 ml collection tubes and 500 µl of wash buffer AW1 added and then centrifuged at 8000 rpm for 1 minute. The flow through together with collection tubes was discarded and the spin column was placed in a new collection tubes and 500 µl of wash buffer AW2 added. A final centrifugation at 14,000 rpm for 3 minutes was performed to remove excess wash buffers. The flow through and collection tubes were discarded and the spin columns placed into sterile 1.5 ml Eppendorf tubes and the bound DNA eluted from the columns using 100 µl of elution buffer AE. The eluted DNA was stored at -30°C until when required.

2.5.3.2 PCR amplification of *T.vaginalis* DNA

A pair of oligonucleotide primers previously described by (Matini *et al*., 2012) were used for PCR amplification. These primers were synthesized by Inqaba biotechnical Industries (South

Africa) and were used to amplify internal transcribed region ITSI/5.8s/ITS2 of ribosomal DNA in *T. vaginalis*. The primer sequences were BTUB1, 5' CGGTAGGTGAACCTGCCGTTGG3' for forward primer and BTUB2, 3'AGTTCAGCGGGTCTTCCTGCG 5'for reverse primer. The PCR was carried out by use of pure Taq Ready–To –Go PCR beads from GE Healthcare UK containing stabilizers, BSA, dATP, dCTP, dGTP, dTTP, 2.5 units of pure Taq DNA polymerase and reaction buffer. The beads were suspended in 25 µl reaction volumes of 2 µl primers of 10mM concentration, 3 µl DNA templates and 20µl nuclease free water. A positive control sample previously confirmed by wet mount microscopy was used in all PCR runs and nuclease free water was used as a negative control. A three-step end point PCR cycling was performed using the Biorad cycler USA, according to the following reaction conditions; initial denaturation at 95°C for15 seconds, followed by 30 cycles of denaturation at 95°C for 45 seconds, annealing at 55°C for 45seconds, and extension step at 72°C for 60 seconds and one final extension at 72°C for 5 minutes.

2.5.4.3 Agarose gel electrophoresis

Following PCR amplification 8 µl of the amplified PCR product was mixed with 2 µl of loading buffer. A molecular weight marker (100 base pair DNA ladder, from Takara Japan), together with the positive and negative controls were run alongside the test samples. The amplified products were then resolved in 2% agarose gel using 1x Tris Borate EDTA as running buffer. The gels were run at 100 volts for 30 minutes then stained with 0.5 µg/ml of Ethidium bromide solution. The gels were visualized under a UV transilluminator and photographed using a gel capture image. The size of the amplified products was then compared with the positive control sample and the DNA molecular weight marker to confirm for positive samples 362bp.

2.6 Data management and analysis

The data were checked for consistency and entered manually into SPSS software version 20. Descriptive characteristics of all the study population were analyzed and presented as percentages. Prevalence of *T. vaginalis* was presented as a proportion with 95% confidence interval and factors associated with *T. vaginalis* were analyzed using chi square test of associations. Odds ratios were calculated and reported as estimates of relative risks of *T. vaginalis* associated with demographic, behavioral and gynecological factors. Sensitivity and specificity of wet mount and conventional PCR was done using contingency table analysis and the findings of this study were presented in graphs and tables.

2.7 Ethical consideration

The research protocol was reviewed and approved by the Kenyatta National Hospital/University of Nairobi Ethical and Research Review Committee (KNH / UON-ERRC). Privacy and confidentiality was maintained throughout and after the research period. Written informed consent was obtained from all willing study participants before data collection. The study subjects benefitted from free, prompt and accurate diagnosis and received their results in the shortest time possible. Free treatment was provided to the sex workers who had the infection immediately after wet mount microscopy.

CHAPTER THREE

RESULTS

3.1 Socio-demographic characteristics of female sex workers visiting SWOP clinics

During the period of study which ran between September 2014 and December 2014, a total of 150 female sex workers who visited three SWOP clinics (Kawangware, Kariobangi and Thika road) were screened for trichomoniasis after obtaining informed consent. The characteristics of the socio demographics of the study participants are shown in Table 1 below. Majority of the sex workers ranged between the ages 18-24 (26%), 25-29 (25%) and 30-34 years (27%) and most of them had primary education (54%), 36% had secondary education, 7% had tertiary education while 3% had not attended school. Overall (74%) of sex workers were single, 8% of the women were divorced and 6% of the sex workers were windowed, married, or separated. Sex work was the sole source of income for the majority of the participants (61%) while 30% of the women owned small businesses and 9% reported being employed. Nearly all (99%) sex workers did not have prior knowledge of Trichomoniasis except only one study participant representing (1%) of the study population.

Table1: Socio-demographic characteristics of sex workers attending Kawangware, Kariobangi and Thika Road SWOP clinics.

Characteristic	Category	N (%) N=150
Age in years (n)=147	18-24	38 (26)
	25-29	36 (25)
	30-34	39 (27)
	35-39	16 (10)
	Over 40	18 (12)
Education (n)=149	None	4 (3)
	Primary	81 (54)
	Secondary	54 (36)
	College	10 (7)
Marital status (n)=143	Single	106 (74)
	Married	8 (6)
	Windowed	9 (6)
	Separated	8 (6)
	Divorced	12 (8)
Other source of income (n)=149	None	91 (61)
	Business	45 (30)
	Employed	13 (9)
Knowledge on STI's		
HIV/ AIDS (n)=147	Yes	103(69)
	No	44(31)
Gonorrhea (n)=149	Yes	137(92)
	No	12(8)
Syphilis (n)=149	Yes	130(87)
	No	19(13)
Trichomoniasis (n)=149	Yes	1(1)
	No	148(99)

Denominator less than 150 due to non response

3.2 Behavioral characteristics of sex workers attending Kawangware, Kariobangi and Thika Road SWOP clinics.

Behavioral characteristics are shown in Table 2 below. Among the 150 participants recruited in this study, (44%) reported engaging in commercial sex with 3-4 male partners per day, 38% had sex with 1-2 sex partners, 10% engaged with 5-6 partners while about 8% had up to 7 partners per day. More than a half (62%) of the sex workers reported using condoms when selling sex. “Always use condom” was reported by 41%, use of condom “most of the time” reported by 29%, use of condom “sometimes” by 22% with 8% of the sex workers reporting not using condoms when having sex. Condom use as reported by the sex workers differed with the type of client. Majority of the sex workers (91%) reported they used condoms with casual clients (a person they have met for the first time) compared to 9% who reported never used condoms with this type of client. There was decrease in condom use with regular client (person they meet frequently) and with regular partner (stable partner). About 66% of the sex workers reported they used condoms with regular clients compared to 34% who never used condoms with these clients. Overall 26% of the sex workers reported they used condom with regular partners while 74% never used condoms with their stable partners. Douching after sex was common among sex workers with 62% reporting the practice. Douching was done mainly using water only by (54%) of the female sex workers while 33% used water and soap, 11% used water and cloth and 1% used salty water and also another 1% used sodium bicarbonate.

Table 2.Behavioral characteristics of female sex workers attending SWOP clinics

Characteristic	Category	N (%)
Number of sex partners per day (n=147)	1-2	56 (38)
	3-4	65 (44)
	5-6	15 (10)
	More than 7	11 (8)
Douching		
Douches after sex(**n=92)**		92 (62)
Douches with	Water only	50 (54)
	Water and Soap	30 (33)
	Water and cloth	10 (11)
	Sodium bicarbonate	1 (1)
	Water and Salt	1 (1)
Condom use (n=148)		
With a casual client		135 (91)
Never		13 (9)
With a regular client		97 (66)
Never		51 (34)
With a regular partner		38 (26)
Never		110 (74)
Frequency of use	*Never*	11 (8)
	Sometimes	32 (22)
	Most time	43 (29)
	Always	62 (41)

Casual client refers to a person they have met for the first time.

Regular client refers to a person they meet on frequently.

Regular partner refers to a permanent partner/stable partner.

3.3. Gynecological characteristics of female sex workers attending SWOP clinics

As shown in table 3 below, women who were included in this study presented with vaginal discharge and for most of them this was not their first time to have the problem. An estimated (33%) reported to have previously had vaginal discharge in the past 2-4 months, 29% in the past 6 months, 26% in the past 5-6months while those who had discharge for the first time were 12%. However, over (62%) of those who complained of vaginal discharge had received treatment in the past. A small proportion (4%) of sex workers reported their male sex partners had complained of urethral discharge while 96% reported their sex partners had never complained of urethral discharge. A greater proportion (45%) of sex workers had 2-3 dependent children, 33% had one child and 13% had no child. About 48% of the women reported not using any family planning method, while 15% reported were using Depo provera, 11% used condoms, 10% were using Norplant, 8% oral pills, with 7% on intra uterine devices and 1% used emergency contraceptive (postinor 2).

Table 3: Gynecological/Obstetrical Characteristics of female sex workers visiting Kawangware, Kariobangi and Thika Road SWOP clinics

Characteristic	Category	N%
Last time to have discharge (n=105)		
	Current month	13 (12)
	2-4 months ago	35 (33)
	5-6 months ago	27 (26)
	>6 months ago	30 (29)
Received treatment for the discharge **(n=128)**		
Yes	Yes	79 (62)
No	No	49 (38)
Partner complain of urethral discharge **(n=150)**	Yes	6 (4)
	No	144(96)
No of children **(n=149)**	none	19(13)
	2-3 children	68(45)
	4-5 children	7(5)
	More than 6	6(4)
	1 child	49(33
Method of Contraceptive used (**n=148**)	None	72(48)
	Oral pills	12(8)
	Norplant	14(10)
	IUD	10(7)
	Postinor 2	1(1)
	Depo	23(15)
	Condom	16(11)

3.4 PCR amplification of *T. vaginalis* DNA

Using the *T. vaginalis* specific primers, PCR amplified a fragment size of 362 bp in positive test samples and also with the positive control sample. No amplification was detected in the negative control sample. The representative gel image in Figure 4 below shows the expected DNA fragment of 362 bp in lane 2, 8 and 11 and was detected in a total of 19 out of the collected 150 HVS samples.

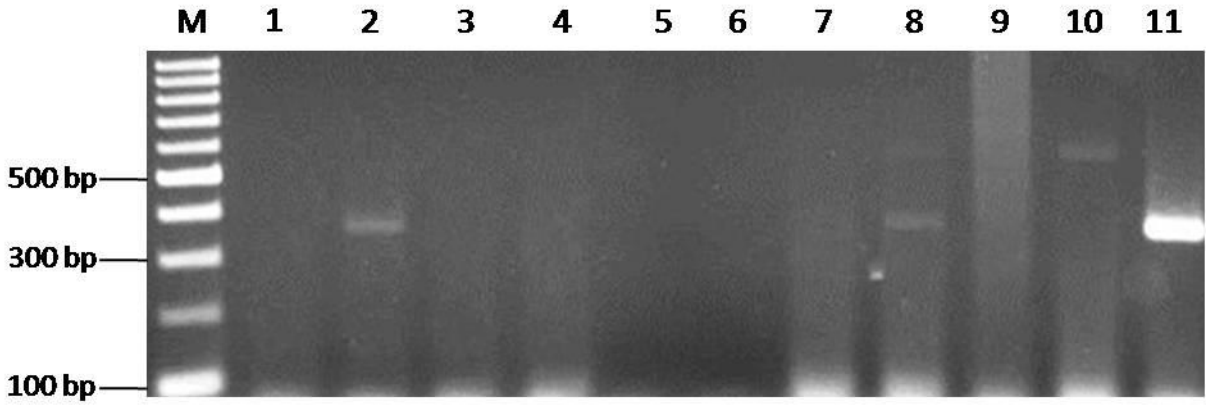

Figure 4: Representative Agarose gel image of the amplified PCR product from *T. vaginalis* DNA from collected high vaginal swabs.

M is the 100 bp molecular weight marker.

Lane 1 is negative control.

Lane 11 is the positive control sample.

Lanes 2 – 10 are the field test samples.

Lane 2 and 8 were samples positive for *T.vaginalis*

Lane 3, 4, 5, 6, 7, 9 and 10 were samples negative for *T.vaginalis*

3.5 Prevalence of *T. vaginalis* by wet mount microscopy and PCR techniques.

Out of 150 specimens collected from female sex workers, the prevalence of *T. vaginalis* was 4.7% (7/150) by wet mount microscopy while PCR detected (19/150) positive samples representing 12.7% (Figure 5). Wet mount microscopy missed 12 samples that were detected by PCR while PCR detected all samples that were positive by wet mount microscopy except one sample.

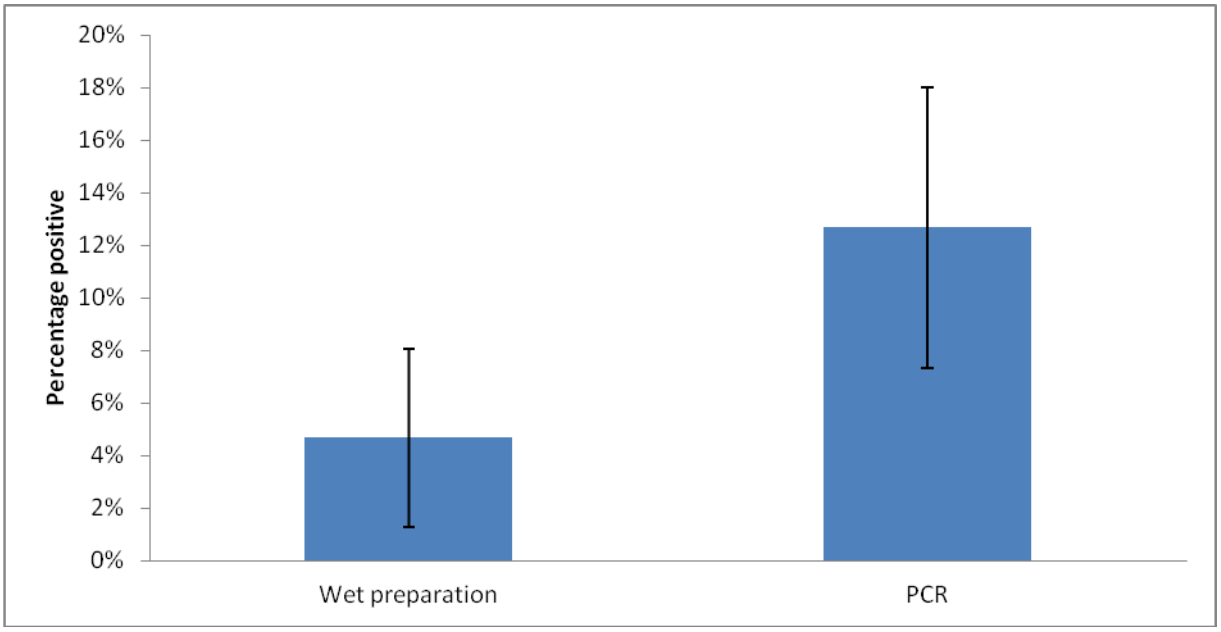

Figure 5: Prevalence of *T. vaginalis* by wet mount microscopy and PCR techniques

3.6 Risk factors associated with *T. vaginalis* among female sex workers attending SWOP clinics.

As shown in Table 4 below, sex workers who never used condoms with their regular client had a higher prevalence of *T. vaginalis* at (23.5%) compared to (7.2%), OR 4.0 (95% CI 1.4-10.8), p=0.008 who did not use condoms. There was no significant association of *T. vaginalis* and use of condoms with casual client and regular partner (p>0.05). Sex workers who reported having 5 or more male sex partners in a day had higher prevalence of *T. vaginalis* (15.4%) compared to those with 3-4 partners (12.3%) and 1-2 partners (12.5%) per day. However, this observed difference was not statistically significant (p>0.05).

Similarly, the prevalence of *T. vaginalis* was slightly higher (14.1%) in sex workers who reported douching after sex compared to their counter parts (10.7%) though the difference was not statistically significant (p=0.547). In addition, *T. vaginalis* prevalence showed an increasing trend associated with increasing years in sex work. Women who had been in sex work for less than a year had a 7.4% prevalence which increased to 23.1% among those who had been engaged in commercial sex for more than 10 years. This trend was however not statistically significant (p>0.05). The prevalence of *T. vaginalis* was higher (16.1%) among sex workers who were HIV positive than those who were HIV negative (11%). However the associated was not significant (p=0.549). All the socio-demographic factors such as age, education and source of income were not associated with presence of *T. vaginalis* among the study participants.

Table 4: Factors associated with positive *T. vaginalis* in sex workers attending SWOP clinics

	***T. vaginalis* PCR**		**Odds ratio (95% CI)**	**P value**
	Positive	**Negative**		
Age group				
18-24years	6 (15.8)	32 (84.2)	1.0	
25-29 years	2 (5.6)	34 (94.4)	0.3 (0.1-1.7)	0.174
30-34 years	6 (15.4)	33 (84.6)	1.0 (0.3-3.3)	0.961
35-39 years	4 (25.0)	12 (75.0)	1.8 (0.4-7.4)	0.430
Over 40 years	1 (5.6)	17 (94.4)	0.3 (0.0-2.8)	0.301
Educational level				
Primary and below	11 (12.9)	74 (87.1)	1.0 (0.4-2.8)	0.936
Secondary and above	8 (12.5)	56 (87.5)	1.0	
Other Source of Income				
None	11 (12.1)	80 (87.9)	1.7 (0.2-14.0)	0.646
Business	7 (15.6)	38 (84.4)	2.2 (0.2-19.8)	0.478
Employed	1 (7.7)	12 (92.3)	1.0	
No of Sex Partners per Day				
1-2 sex partners	7 (12.5)	49 (87.5)	1.0	
3-4 sex partners	8 (12.3)	57 (87.7)	1.0 (0.3-2.9)	0.974
5 and more sex partners	4 (15.4)	22 (84.6)	1.3 (0.3-4.8)	0.722
Uses condom with casual client				
No	1 (7.7)	12 (92.3)	0.5 (0.1-4.4)	1.0
Yes	18 (13.3)	117 (86.7)	1.0	
Uses condom with regular client				
No	12 (23.5)	39 (76.5)	4.0 (1.4-10.8)	0.008
Yes	7 (7.2)	90 (92.8)	1.0	
Uses condom with regular partner				
No	15 (13.6)	95 (86.4)	1.3 (0.4-4.3)	0.782
Yes	4 (10.5)	34 (89.5)	1.0	
Douching after Sex				
No	6 (10.7)	50 (89.3)	1.0	
Yes	13 (14.1)	79 (85.9)	1.4 (0.5-3.8)	0.547
Duration in Sex Work				
Less than 1 year	2 (7.4)	25 (92.6)	1.0	
2-4 years	9 (13.0)	60 (87.0)	1.9 (0.4-9.3)	0.442
5-9 years	4 (11.4)	31 (88.6)	1.6 (0.3-9.5)	0.598
Over 10 years	3 (23.1)	10 (76.9)	3.8 (0.5-25.9)	0.180
HIV status				
Positive	5 (16.1)	26 (83.9)	1.4 (0.5-4.3)	0.549
Negative	14 (11.0)	104 (88.1)	1.0	

3.7 Duration of motility of *T. vaginalis* following sample collection.

All (7/150) high vaginal swabs which were positive by wet mount microscopy were preserved in normal saline and re- examined under the microscope for motility of trichomonads after 30 minutes and then after every one hour interval for a maximum of 5 hours. The same swabs were transported to University of Nairobi Institute of Tropical and Infectious diseases (UNITID) laboratory for examination by a second independent microscopist. Motility of *T. vaginalis* was observed by movement and vibration of flagella and undulating membrane. In all the samples motility of *T. vaginalis* reduced after every subsequent hour and by the 5^{th} hour almost all the trichomonads were immotile and could only be identified by vibration of the flagella and undulating membrane.

3.8 Sensitivity and specificity of wet mount microscopy

As shown in Table 5 below, wet mount microscopy correctly detected 6 positive *T. vaginalis* out of the 19 confirmed with PCR. On the other hand, 130 samples were negative for *T. vaginalis* out of 131 true negatives confirmed through PCR. Wet mount microscopy was 31.6% sensitive and 99.2% specific. Positive predictive value was 85.7% (1 sample was positive by wet mount but negative by PCR) negative predictive value was 90.9%.

Table 5: Comparison of wet mount microscopy and PCR techniques in detection of *T. vaginalis* in symptomatic female sex workers attending SWOP clinics

Result	**PCR**		**Total**
	Positive	Negative	
Wet prep			
Positive	6	1	**7**
Negative	13	130	**143**
Total	**19**	**131**	**150**

CHAPTER FOUR

DISCUSSION, CONCLUSION AND RECOMMEDATION

4.1 DISCUSSION

In the current study, the prevalence of *T. vaginalis* among female sex workers visiting SWOP clinics was 12.7% by PCR and 4.7% by wet mount microscopy. These findings were comparable with other previous studies conducted in Kenya such as a study that was conducted in Kisumu by (Vandenhoudt *et al*., 2013) which reported a decline in the prevalence of *T. vaginalis* among sex workers from 45.2% in 1997 to 13.6% in 2008. The results also concurred with another study (MARPS Surveillance-Kenya 2012) that reported a prevalence of *T. vaginalis* at 10.3% by PCR among female sex workers. However the prevalence in the current study is higher than results by (Gomih-Alakija *et al*., 2014) that reported the prevalence of *T. vaginalis* at 7.2% and conducted his research in one of the SWOP clinic shared in the current study (Kariobangi). The difference between this study and Gomih –Alakija may be attributed to various factors. First, it could have been due to increase in *T.vaginalis* prevalence in the last 3 years after Gomih-Alakija research which was carried out between August 2009 and March 2011. Secondly could also be as a result of selection criteria of the study participants since the current study recruited sex workers who presented with vaginal discharge a factor that was not considered in the previous study. Thirdly it could be due to inclusion of study subjects from two other study areas Thika road and Kawangware clinics which were not included in the previous study.

The Prevalence of *T.vaginalis* in the current study was lower compared to previous studies conducted in other parts of the world that used PCR technique in the diagnosis of *T. vaginalis* among sex workers. In a cross sectional study conducted in Papua New Guinea higher

prevalence of *T. vaginalis* was reported at 41% (Bruce *et al*., 2010). In Ndola Zambia, the prevalence of *T.vaginalis* among commercial sex workers was 33.2% (Crucitti *et al*., 2010). Similarly in Manado Indonesia the prevalence of *T. vaginalis* among sex workers who were recruited at their work place was 22.6% (Mawu *et al*., 2011). This could be attributed to difference in geographical location of the study areas as prevalence of *T.vaginalis* has been reported to vary in different countries (Kissinger, 2015b).It could also be due to the fact that people are getting more informed on issues of sexuality and have become careful by using protective devices to avoid infections. In the current study majority 62% of the women reported using condoms while selling sex hence the reduction in the prevalence of Trichomoniasis. Furthermore, the sex workers in this study were aware of STI's as assessed using a questionnaires as follows (92% Gonorrhea, 87% syphilis, and 69% HIV AIDS) and this could have helped them to be careful in their work. Another reason could be the type of PCR used in the studies. For instance in Manado Indonesia the specimens were analysed using multiplex PCR and this could have been more powerful compared to the current study that analysed specimens using single PCR. However the prevalence of *T. vaginalis* in this study was higher compared with a study that was conducted in Philippines that reported a low prevalence of 6.8% among sex workers (Queza and Rivera, 2013). The explanation for this could be due to difference of the study subjects as Queza and Rivera recruited both males and females sex workers who attended periodic screening for Trichomoniasis and most likely they had no symptoms of the disease while in this study we recruited female sex workers who presented with vaginal discharge hence the higher prevalence of Trichomoniasis.

Compared to studies which also detected *T. vaginalis* by wet mount microscopy among sex workers, the current study results of 4.7% concurred with a study conducted among women

selling sex in Lahore, Pakistan that reported a prevalence of 5.1% (Khan *et al*., 2011). However, low prevalence of *T.vaginalis* was found in the current study compared to studies conducted in Bangladesh and Mongolia among female sex workers that reported a prevalence of 45.5% and 28% respectively (Rahman *et al*., 2000, Hagan and Dulmaa, 2007). The low prevalence of *T. vaginalis* in this study compared to other previous studies was as a result of recruitment of sex workers who were already enrolled in the SWOP clinics where free education, screening of sexually transmitted infections, treatment and prevention services were offered.

The study found out that, prevalence of *T. vaginalis* among female sex workers was significantly associated with inconsistent condom use with regular clients (person they meet frequently).Sex workers who never used condoms with their regular clients had higher prevalence (23.5%) of Trichomoniasis than those who used condom (7.2%) with the same clients and this was statistically significant (P=0.008). Due to the frequency of meeting with the regular clients the sex workers could have ignored use of condoms hence getting infected. Furthermore women who had many sex partners 5 or more had a higher likelihood of being infected (15.4%) than those who had 3-4 (12.3%) partners and 1-2 (12.5%) partners and these findings were in agreement with other previous studies (Verteramo *et al*., 2008; Fernando *et al*., 2012; Perla *et al*., 2012).

Similar with other studies (Behets *et al*., 2005, Verteramo *et al*., 2008) the prevalence of *T. vaginalis* was also associated with duration of prostitution as the prevalence was found to increased with increasing years in sex work. Women who had more than 10 years in sex work had a higher prevalence of *T. vaginalis* at 23.1% compared to those who had less than a year (7.4%). These findings implied that the commercial sex workers who had many years of selling

sex were older, used condom less and may be, douched more. In addition, the sex workers could have been carriers of the parasite or possibly suffered re-infections from their sex partners.

Our findings concur with studies conducted in other parts of the world which have reported an association of vaginal douching with prevalence of *T. vaginalis* (Sutton *et al*., 2007).Vaginal douching reduces the density of normal vaginal flora and thus exposing the reproductive area to colonization by STI's including Trichomoniasis and thus in the current study sex workers who douched were more likely to have high prevalence of *T. vaginalis* (14%) than those who never douched (11%). Likewise the current study found higher prevalence of *T. vaginalis* in HIV infected women (16.1%) than those who were HIV negative (11%) though the association was not statistically significant, and this observation concurred with other studies that reported a positive association between *T. vaginalis* and HIV infection (Sorvillo *et al*., 2001, McClelland *et al*., 2005, Van Der Pol *et al*., 2008).However unlike other studies(Verteramo *et al*., 2008, Fernando *et al*., 2012) our study reports that socio demographic characteristics such as age, education and source of income where not associated with *T.vaginalis* infections.

Diagnosis of *T. vaginalis* infection mainly relies on detection of trophozoites by wet mount microscopy because it is rapid and inexpensive. Trophozoites of *T. vaginalis* are missed by this method especially when the infection is low and in addition it is not possible to differentiate *T. vaginalis* trophozoites microscopically from other flagellates that contaminate the sample during collection for example *Trichomonas hominis.*

In this study wet mount microscopy was carried in less than 30 minutes following sample collection but the proportion of positive samples detected by this method was low with only 6 confirmed positive compared to 19 detected by PCR. The sensitivity and specificity of wet

mount microscopy was 31.6% and 99.2% respectively. Our findings were comparable with a recent study that was conducted in UK by (Nathan *et al*., 2015) which reported the sensitivity of wet mount microscopy at 38% and with another study by (Patil *et al*., 2012) that reported a higher sensitivity 60% and specificity of 100%. The sensitivity and specificity of a test method depends on the gold standard used. In this study this low sensitivity of wet mount was as a result of comparison with PCR which is a very sensitive method. Surprisingly 1 sample which was positive by wet mount microscopy was not detected by PCR and this could have occurred if the infection was low resulting to one swab picking trophozoites and the other swab not picking trophozoites or may be the two swabs collected specimens from different vaginal areas. In addition the specimen might have been contaminated by other intestinal flagellates which could not be detected by the PCR primers used.

Little is known on motility of *T. vaginalis* in collected HVS and to the best of my knowledge; this was the first research in Kenya that did this investigation. The findings of this study are in agreement with past reports on immediate examination of the collected specimen for maximum motility of the trichomonads (Kingston *et al*., 2003, Stoner *et al*., 2013). In this current study, when the swabs were preserved in normal saline immediately after collection, motility decreased after every subsequent hour and by the 5^{th} hour no motility was observed and the trophozoites could only be identified by vibration of the flagella and undulating membrane. One limitation of our finding was that, we had few positive samples (7) to detect motility. However, a larger sample size would likely improve the accuracy of the present findings.

4.2 CONCLUSION

The prevalence of *Trichomonas vaginalis* among symptomatic female sex workers is relatively high as indicated by results by PCR and there is underreporting/underestimation of prevalence of *T. vaginalis* in our local setting by wet mount microscopy. This study has confirmed wet mount technique is insensitive in detecting *T.vaginalis* as reported by other similar studies and many positive cases are missed by this method. From the study, PCR was more powerful tool and can be looked into as a future diagnosis tool for screening of *T.vaginalis*. Moreover, the study has noted that collected HVS can be preserved in normal saline incase of delay in examination.

4.3 RECOMMEDATION

1) For effective screening of *T. vaginalis* highly sensitive and specific techniques should be considered such as PCR for detecting of infections in both symptomatic and asymptomatic patients.

2) Clinicians should be advised on preservation of HVS in normal saline immediately after collection in case of delay in examination.

3) Creation of awareness for example education posters, encouraging checkups to increase knowledge on Trichomoniasis among the sex workers will help to control the spread of this infection.

4) Further investigation on motility of *T. vaginalis* in collected HVS using a larger sample size can be considered.

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APPENDIX 1

INFORMATION AND CONSENT FORM

PREVALENCE OF *T. VAGINALIS* IN RELATION TO SEXUAL BEHAVIOUR AMONG FEMALE SEX WORKERS VISITING SWOP CLINICS IN NAIROBI, KENYA

INTRODUCTION

Hello. My name is **Felista Wayua Muthini** from the University of Nairobi. I am conducting a research to determine the prevalence of *Trichomonas vaginalis* in relation to sexual behavior among female sex workers. The purpose of this consent form is to give you information that will help you decide whether to be in the study or not. Please read the form carefully .You may ask questions on anything that is not clear about the study. When we have answered all your questions, you can decide if you want to be in the study or not.

Purpose of the research

The purpose of this study is to find out the prevalence of *Trichomonas vaginalis* which is a sexually transmitted parasite. In addition, i also want to know how sensitive is the locally available technique used in diagnosis of this parasite in Kenya and also find out the duration of time required to examine collected vaginal discharge. The results which I will get will help to reduce *Trichomonas vaginalis* infections and also improve its diagnosis.

Type of Research Intervention

This research will involve one visit, participation will include a questionnaire and collection of vaginal discharge by a trained nurse using a sterile swab .The collected swab will not be labeled with your name instead a number will be used.

Participant Selection

You are invited to take part in this research if you are aged 18 years and above , you have vaginal discharge and you have not been on treatment for the last 30 days.

Voluntary Participation

Your participation in this research is entirely voluntary. It is your choice whether to participate or not. If you choose not to participate all the services you receive at this clinic will continue and nothing will change.

Procedures

This is what will happen if you consent to participate in this study, you will be asked questions by the researcher about yourself. After answering these questions you will be send to the Doctors room. Here the procedures of High vaginal swab collection will be explained to you by a trained nurse. If you will agree to participate, two high vaginal swabs will be collected. One swab will be processed immediately and you will be given your results. If the results are positive you will be treated immediately. If the results are negative you will be requested to come back after one week to collect your PCR results. I would like to invite you to participate because the results obtained from this study will help other people like you in improving diagnosis of *Trichomonas vaginalis* in the hospitals. Please be assured that the information that you will give me will not be linked to any names or other identifying information.

Risks

I am asking you to share with me some very personal and confidential information, and you may feel uncomfortable talking about some of the issues. You do not have to answer any question or

take part in the discussion if you don't wish to do so. You do not have to give me any reason for not responding to any question, or for refusing to take part in the questionnaire. The collection of the specimen may also be uncomfortable for you and you are free to accept or refuse the procedure.

Benefits

There will be no monetary benefit to you, if your results will be positive, you will be given free treatment immediately. Your participation will help us in improving diagnosis of this parasite in Kenya.

Confidentiality

The information that we collect from this research project will be kept private. Any information about you will be reported anonymously and no names will be used.

You can ask me any questions about any part of the research study, if you wish to. Do you have any questions?

Whom Do I Call if I Have Questions or Problems?

For questions about the study or a research-related injury, call or contact Felista Wayua Muthini, Department of Medical Microbiology, University of Nairobi. Tel: 0723657803 or my supervisors Dr. Odongo Tel 0722984090, Dr. Oyugi Tel 0713898564 or Dr. Kimani Tel 0733719711.

For questions about your rights as a research participant contact,

The Secretary, Kenyatta National Hospital/University of Nairobi Ethics and Research Review Committee Tel: 726300-9, Fax: 725272, Email: uonknh_ erc@uonbi.ac.ke

Physical Address

KNH School of Pharmacy, UON behind the KNH Dental Clinic

P.O Box 20723-00202 Nairobi

SUBJECT'S STATEMENT

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions I have asked have been answered to my satisfaction. I consent voluntarily to be a participant in this study

Name of Participant _____

Signature of Participant _____

Date _____

APPENDIX 2

QUESTIONNAIRE

NB: This information will be communicated orally in English, Swahili or other Kenyan dialect of potential participant's preference

Participant no._____ Date of interview_____

Name of the SWOP clinic_____

1. Age (a) 18-25 years (b) 25-29 years

(c) 30-34 years (d) 35-39 years

(e) Over 40 years

2. Highest educational level attained (tick one)

(a)None (c) Primary

(b)Secondary (d) College (post secondary)

3 .Marital status (tick one)

(a) Single (b) Married

(c)Widowed

4. Other source of income

(a) None (b) Business (c) Employed

5. How many sex partners per day?

(a) 1-2 (b) 3-4

(c) 5-6 (d) 7 and more

6. Average no of sex partners per week

(a) 1-2 (b) 3-4

(c) 5-6 (d) 7 >more

7. What methods of family planning do you use?

(a) None (b) Pills

(c) Norplant (d) IUD

8. When do you use condom during sex

(a)Never (b) With a casual client (person they have met for the first time)

(c)With a regular client (Person they meet regularly)

(d)With a regular partner (like a permanent partner)

9. % use of condoms?

(a) Never (b) Sometimes

(c) Most time (c) Always

10. Do you douche after sex?

(a)Yes (b) No

11. If yes, why

(a)To be clean (b) to prevent infection (c) Other

12. Douche with what

(a)Water and soap (b) water only

13. Presenting illness

(a)Vaginal discharge (b) other

14. If the answer is vaginal discharge. Is this your first time to have this discharge?

(a) Yes (b) No

15. if no, when did you last have vaginal discharge?

(a) Current month (b) 2-4 months ago

(c) 5-6 months ago (d) more than 6 months ago

16. Did you receive treatment for the discharge?

(a)Yes (b) No

17. Does any of your partners complain of urethral discharge?

(a)Yes (b) No

18. What diseases can you get through sexual activity?

(a) HIV and AIDS (b) Gonorrhea

(c)Syphilis (d) Trichomoniasis (e) Other

19. How long have you been is sex work

(a) Less than 1 year (b) 2-4 years

(c) 5 -9 years (d) over 10 years

20. How many children do you have?

(a) None (b) 1

(c) 2-3 (d) More than 6