

Identification of *Trichomonas vaginalis* Parasites In Commercial Sex Workers Conducting Examinations At The Mawar Clinic PKBI Bandung, Indonesia

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ABSTRACT

Trichomoniasis is a sexually transmitted infection (STI) caused by the pathogenic *Trichomonas vaginalis* protozoa parasite found in the female genitourinary tract and can cause vaginal discharge, in severe conditions, vaginal inflammation will occur. *Trichomoniasis* has a high risk of infecting groups that often have multiple partners, such as Commercial Sex Workers (CSWs). This study aims to determine the presence or absence of *Trichomonas vaginalis* parasites in the urine sample of Commercial Sex Workers who conduct examinations at the Mawar Clinic PKBI Bandung. The research method used is observation. The sample is the urine of a prostitute who performs an examination at the Mawar Clinic PKBI Bandung, Indonesia. The examination was carried out in two ways, namely the wet method and the Giemsa staining method, which was carried out in the Rajawali Bandung Institute of Health laboratory. The results showed that out of 30 samples, 11 urine samples of CSW were infected with *Trichomonas vaginalis*. The conclusion of this study is that *Trichomonas vaginalis* parasites were found in urine samples of prostitutes who were examined at the Mawar Clinic PKBI Bandung.

KEYWORDS

Trichomonas vaginalis; Sexually Transmitted Infection (TFI); Commercial Sex Workers

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Introduction

Trichomoniasis is a sexually transmitted infection (STI). *Trichomoniasis* has a high risk of infecting groups of people who often change partners, one of these groups is Commercial Sex Workers (CSWs). Commercial sex workers are part of sex activities outside of marriage which are marked by the satisfaction of various people by involving several men who are done for money and serve as a source of income (Koentjoro, 2004). The prevalence of *Trichomonas vaginalis* in women commercial sex workers is 50-60%, while in the general population, women are only 5-10%. These sex workers can transmit the *Trichomonas vaginalis* parasite to their customers (Zulkoni, 2011)

Trichomoniasis is a disease caused by infection with *Trichomonas vaginalis*, a pathogenic protozoan that is commonly found in the female genitourinary tract and can cause vaginal discharge (flour albus) or leukorrhoea and in more severe conditions, inflammation of the vagina or vaginitis will occur. Whereas in men, *Trichomoniasis* infection is found in the urethra, which causes non-gonorrhoea urethritis. *Trichomoniasis* is often found in conjunction with other STIs, such as HIV/AIDS or gonorrhoea.

The results obtained from 50 sex workers, 11 people (22%) were infected with the protozoa *T. vaginalis* with the most sexual characteristics of sex workers based on age, namely 26-35. years as many as 27 people (54%), the most being equivalent to Junior High School age as many as 22 people (44%), the length of being a prostitute is 7-12 months, namely 18 people (36%), the highest number of customers is 3 people per day as many as 14 people (30%), the most marital status was ever married as many as 23 people (46%), the most from the island of Java as many as 41 people (82%), customers CSW using condoms as many as 37 people (74%), CSW who do not use vaginal antiseptic every day as many as 32 people (22%), sex workers often experience vaginal discharge as many as 32 people (64%) and experience itching and heat as many as 6 people (12%) while the characteristics of the level of knowledge of sex workers about the dangers of infection with *T. vaginalis* are mostly eight people (16%).

In women, the transmission of this *Trichomoniasis* disease can occur directly or indirectly. The direct transmission is through sexual intercourse, while the indirect method is the use of public facilities that are not kept clean, such as toilet seats, swimming pools, underwear, and contaminated river water. Whereas in men usually only direct transmission occurs through sexual intercourse (Lovarita, 2012)

Based on previous research conducted by (Pratomo et al., 2011), *Trichomoniasis* infection from 30 samples of CSWs localized by Sunan Kuning Semarang was 6.67%. While in another study conducted by Khairiyah et al (2013), *Trichomoniasis* infection in 35 samples of CSWs was 17.14%.

The Bandung City Government, through data collection conducted by the Bandung City Social Service in 2012 verified the number of commercial sex workers in Bandung as many as 319 people. This data is different from the data held by the Pamong Praja Police Unit (Satpol PP) which was reported by a local newspaper which noted that the number of prostitutes in 2012 reached more than 1,000 people. This data is supported by a data collection/survey conducted by the Mawar PKBI Clinic (a clinic that plays an active role in counseling for CSWs) which shows similar data in the same year. In 2013, the number of sex workers who were in active counseling at the Mawar PKBI Clinic was 130 people. (Silvie, 2015). The latest data from the Mawar Clinic in 2017-2018 showed the number of positive STI patients was 250 people, while in 2018-2019 the number of positive STI patients was 350 people, this data shows that there has been an increase in STI positive patients who underwent examinations at the Mawar Clinic PKBI experienced an increase of about 100 people every year.

Based on the explanation above, the researchers took the initiative to conduct *Trichomonas vaginalis* research on urine samples of sex workers who were examined at the Mawar Clinic of PKBI Bandung. So far, the laboratory tests used to diagnose *Trichomoniasis* are vaginal secretions (vaginal swabs) and urethral secretions (Neneng Syarifah, 2009). Examination of vaginal secretions is a follow-up examination as confirmation of samples with positive results on the initial examination (screening) to avoid false positive results. Therefore, an initial examination (screening) is needed as an alternative that can support the diagnosis of *Trichomoniasis*, namely by using urine sediment (Lovarita, 2012). In addition, there has never been a *Trichomoniasis* study in the urine samples of CSWs at the Mawar Clinic of PKBI Bandung.

Literature Review

Trichomoniasis is a protozoan infection attacks the lower urogenital tract in both men and women and caused by the parasite *Trichomonas vaginalis*, this disease is usually transmitted through sexual intercourse. Humans are the host for this parasite.

Garcia and Buckner (1996) (Khairiyah et al., 2013), mentions that *Trichomonas vaginalis* is a eukaryotic organism that includes the Mastigophora group, have flagella, with the order Trichomonadida which contains more than 100 species, most of the *Trichomonas* are commensal organisms in the mammalian intestine and bird. There are 3 species that are often found in humans, namely: *Trichomonas vaginalis*, *Trichomonas tenax* and *Trichomonas hominis*.

Important aspects that need to be considered in order to avoid *Trichomoniasis* is an individual behavior in terms of behavior sex and personal hygiene. The basis of treatment is to improve the condition of the vagina by cleaning the vaginal mucosa and using peros drugs and locally such as metronidazole. (Sutrisno, 2017)

Method

This type of research is observational reasearch. The researcher wanted to know whether or not the *Trichomonas vaginalis* parasite was present in the urine samples of sex workers who were examined at the Mawar Clinic of PKBI Bandung. Examination of the *Trichomonas vaginalis* parasite was carried out in two ways, namely the wet method, followed by Giemsa staining.

Method of sampling

First to take a urine sample from the prostitutes who conduct the examination at the Mawar Clinic of PKBI Bandung. The collected urine is urine while and the urine sample used for this study does not use preservatives, because it will be immediately examined at the Rajawali Health Institute Laboratory. Then prepared a sterile urine container with a size of 40cc. Urine is accommodated into the urine pot up to the 15cc limit mark, the rest is discarded. After that the urine sample is then taken to the Rajawali Health Institute Laboratory for examination, the time span is 1-3 hours after urine collection.

Method of sampling

The samples that have been obtained are immediately used in wet preparations, after direct examination (wet preparations) and *Trichomonas vaginalis* are found in the samples, then they are painted using methanol and Giemsa to help identify the morphology of *Trichomonas vaginalis*. The staining technique used is Giemsa staining.

Direct way (Wet way): Urine was transferred from the reservoir into a centrifuge tube to be centrifuged at a low speed of 1500-2000 rpm for 5 minutes. After centrifugation the supernatant was discarded. Then the precipitate is homogenized and physiological NaCl is added, pour 1 drop on the object glass and then cover with a cover glass. Urine was examined using a microscope with a magnification of 40x and then 1000x. *Trichomonas vaginalis* can be observed with characteristics of small, transparent, about the size of a white blood cell and moving quickly in in circles. After that, the sample was examined using a medium magnification of 40x and then 100x to observe *Trichomonas vaginalis* (Khairiyah et al., 2013)

Giemsa Painting: One drop of homogeneous urine sediment placed on a glass object and then made a thin exposure and allowed to dry, after drying, fixation with methanol for 1 minute. Before be painted, the Giemsa paint is diluted 1:10 (1 ml Giemsa solution + 9 ml aquadest). The preparation is placed on a painting rack, and poured with diluted Giemsa paint, let stand for 10 minutes. Then wash with running water, removed and cleaned the bottom of the object glass then place it on the drying rack, let it dry. After drying, the preparation was observed microscopically with a 100x objective and oil immersion (Lovarita, 2012)

Instruments and Materials

The tools used in the examination include Deck glass, Stick / toothpick, Microscope, Glass object, Drop pipette, Urine pot, Centrifuge, Centrifuge tube. The materials used in this examination are Giemsa paint, Methanol, Oil immersion, Physiological NaCl, CSW urine sample.

Data analysis

The data obtained from the examination is arranged in the form of a table of data obtained and then the percentage is calculated. From examination of 30 urine samples, the following result were obtained: urine sample of prostitutes who conduct examinations at the Mawar Clinic PKBI Bandung the percentage of urine samples that are positive 37% and negative 63%.


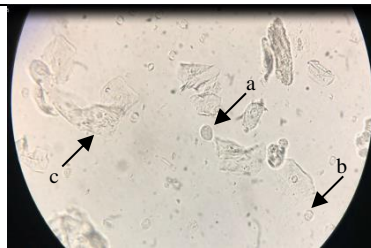
Results


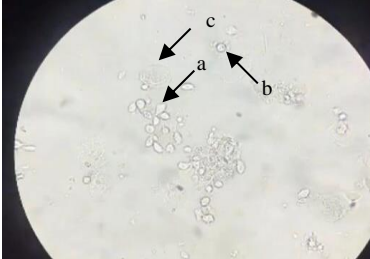

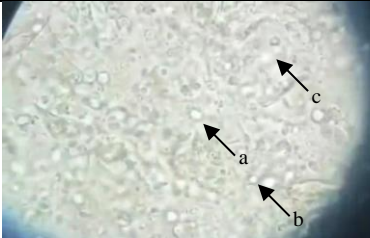
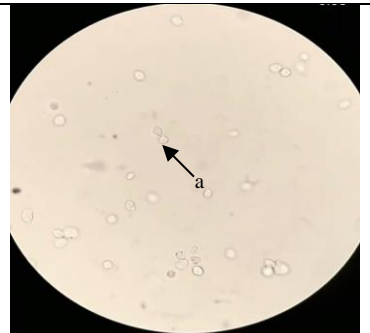

The study, which was conducted on July 21 to August 7, 2020, of 30 samples of urine of Commercial Sex Workers who conducted an examination at the Mawar Clinic of PKBI Bandung, the results of the finding of *Trichomonas vaginalis* in the urine of respondents were 11 people. Following are the results of the research conducted, the results obtained are interpreted at the Table 1 and Table 2.

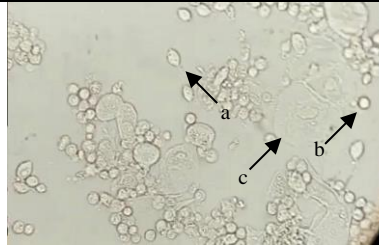
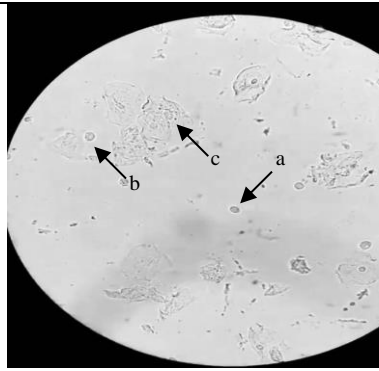
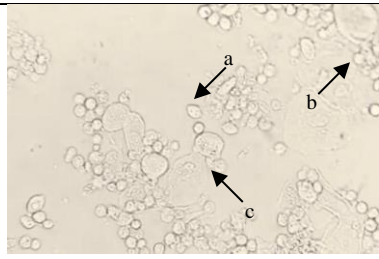
Table 1. Results of Examination of *Trichomonas vaginalis* Wet Preparation

The incidence of <i>Trichomoniasis</i>	Total (n)	Persentase
Found <i>Trichomonas vaginalis</i>	11	37%
No Found <i>Trichomonas vaginalis</i>	19	63%
Total	30	100%

Table 2. Observations using a microscope

No.	Sample Code	Result	Microskopik
1.	01	(+)	
2.	12	(+)	

3.	15	(+)	
4.	17	(+)	
5.	20	(+)	
6.	21	(+)	
7.	22	(+)	
8.	23	(+)	

9.	25	(+)	
10	26	(+)	
11	28	(+)	

Information::

a : *Trichomonas vaginalis*

b : Leukocyte cells

c : Epithelial cells

Wet Test Results

The results of the wet examination of a positive CSW urine sample for *Trichomonas vaginalis* in the field of view seen under a 100x magnification microscope, it was seen that the parasite was in the middle between leukocyte cells and epithelial cells with morphological characteristics of an asymmetrical shape like a pear, with the size of a pear. $\pm 15-18 \mu$, has 1 flagellum in the posterior, and has 3 flagella in the anterior (in the picture less visible), moves in circles, is fast, and jerks

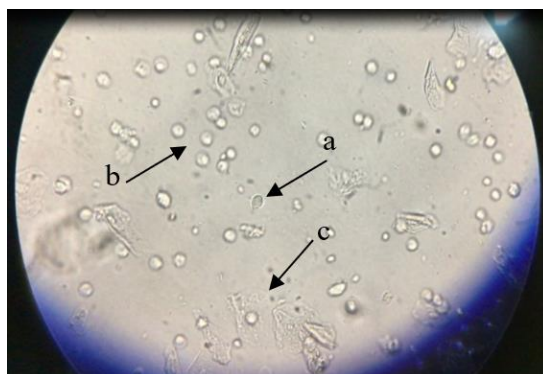


Figure 1. Field View of Wet Preparation of Positive CSW Urine Samples for *Trichomonas vaginalis* (a: *Trichomonas vaginalis*; b: leukocyte cell; c: epithelial cell)

The results of the wet method on urine samples of CSWs that were negative for *Trichomonas vaginalis* were seen under a microscope with a magnification of 100x, the parasites were not visible in the entire visual field examined, in the negative urine field, only cells such as leukocytes, epithelial cells, and erythrocytes were seen and also no other bacteria were found.



Figure 2. Field View of Wet Preparation of Negative *Trichomonas vaginalis* CSW urine samples

Information:

a : Leukocyte cell

b : Epithelial cell

Results of the Examination Method of Coloring

The results of the staining method using Giemsa urine samples that were suspected to be positive for *Trichomonas vaginalis* in the field of view viewed under a microscope at 100x magnification showed that there were two appearances of the parasite with morphological characteristics of a pear-like shape, with a size of $\pm 20-23$, there were -Each nucleus in the anterior, there is 1 flagellum in the posterior but the flagellum in the anterior is less clearly visible, the trophozite stage, and the parasite is silent or dead.

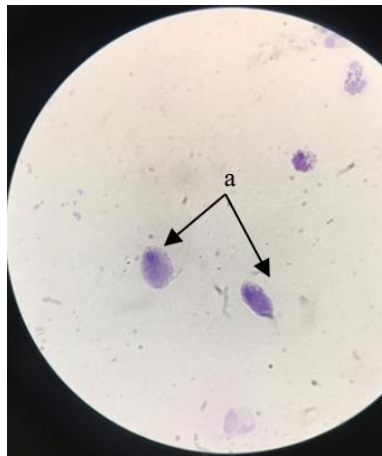


Figure 3. Field of view staining of positive CSW urine samples for *Trichomonas vaginalis* (a : *Trichomonas vaginalis*)

The results of the staining method using Giemsa urine samples that were negative for *Trichomonas vaginalis* in the visual field seen under a microscope at 100x magnification, did not show any parasites with morphological characteristics such as *Trichomonas vaginalis*. In all visual fields examined, only leukocytes and epithelial cells were seen.

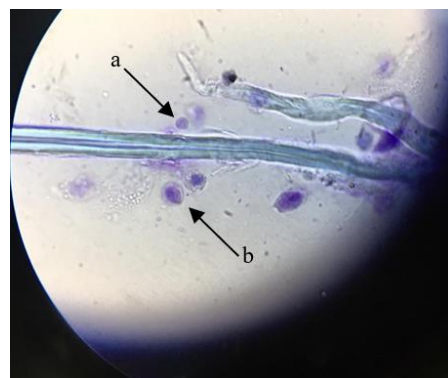


Figure 4. Field of view of negative *Trichomonas vaginalis* CSW urine staining (a : Leukocyte Cell; b : Epithelial Cell)

Discussion

This research was conducted starting from a survey to the Mawar Clinic PKBI Bandung which is located on Jl. Pasir Kaliki No.26, or to be precise, opposite 23 Paskal Hypersquare Mall, and asked for permission to request 30 samples of CSWs urine samples by providing a research permit to the Mawar Clinic. After the Mawar Clinic gave permission for the study, the researchers began to take urine samples from women who were commercial sex workers who did an examination at the Mawar Clinic at PKBI Bandung from 21 July to 7 August 2020.

Based on the results of research that has been carried out from 30 urine samples of female commercial sex worker patients who conducted an examination at the Mawar Clinic of PKBI Bandung, it was found that the trophozoite parasite *Trichomonas vaginalis* was positive in 11 respondents and negative in 19 respondents.

The distribution of CSW respondents based on the results of the questionnaire according to length of service, the majority were CSWs who had worked for >13 months 67% and those who worked <12 months 33%. partners, the distribution of CSWs who last had sex with >2 people was 60% and those who had sex with only 1 person was 40%, so according to Zulkoni (2011) the prevalence of infection with Trichomoniasis is in groups of people who frequently change partners such as sex workers. Which is 50-60% while in the general female population it is only 5-10%.

The distribution of CSW respondents based on the results of a questionnaire based on their knowledge of *Trichomonas vaginalis*, showed that 97% of CSWs did not know about the *Trichomonas vaginalis* parasite that can cause Trichomonas infection. as quoted from the CDC (2015), Trichomoniasis infection can cause serious disease complications such as increasing HIV transmission, vaginal inflammation or vaginitis in women and urethritis in men, causing premature birth or low birth weight babies, causing cervical cancer, and causing pelvic inflammatory disease in women.

The distribution of CSW respondents based on the results of the questionnaire according to age, respondents who were positively infected with Trichomoniasis were in the age range of 20-30 years, namely 77% and at the age of 31-49 years 23% because that age was in the sexually productive period, while the age range <19 years and >50 years, cases are rare. According to the Department of Parasitology FKUI (2008), in women this parasite is more often found in the age group of 20-49 years, decreases in young and old age and rarely in girls.

Factors that can cause a person to become infected with Trichomoniasis include lack of attention to the following things such as personal hygiene (Personal Hygiene). The distribution of CSW respondents based on the results of the questionnaire according to Personal Hygiene, namely, the use of condoms when having sex, only 27% of sex workers who always used condoms and 73% of those who didn't or rarely used condoms. According to WHO (2007), condoms are effective in reducing, but not completely preventing, although using a condom, the risk of infection is very possible, especially if you don't use a condom, many other sexually transmitted infections can be transmitted, so it is highly recommended to use a condom every time you have sex.

Other factors that can affect Trichomoniasis infection are the use of vaginal antiseptic soap that is not suitable so that it can change the vaginal pH to be unstable and can cause *Trichomonas* parasites to multiply in the vagina, the distribution of sex workers who use vaginal antiseptic soap is 40% and those who do not use it is 60%. In addition, there are also factors from the use of towels/clothes simultaneously and the use of public facilities simultaneously such as toilet seats, towels/pants/shared clothes, and swimming pools because according to Zulkoni (2010), the *Trichomonas vaginalis* parasite that attaches to surrounding objects can live for 45 minutes. The distribution of CSW respondents based on the questionnaire results according to the use of the facilities simultaneously, namely, 97% of CSWs who often use the toilet seat and 3% who did not use, 3% of CSWs who frequently used shared towels/pants/clothes, and 47% of CSWs who did not use common clothes. The CSWs who often swim in public swimming pools are 50% and those who don't are 50%.

Distribution of CSW respondents based on the results of questionnaires according to routine urine screening tests, namely, those who have had a urine test 97% of them have never done it, only 3% have ever done a urine test as a screening, even though routine urine tests are very important to do as an early detection effort. A person's symptoms indicate a disease, not just a disease of the genital tract.

Based on the results of the questionnaire also showed that all respondents who indicated positive *Trichomonas vaginalis* had not received or had never undergone treatment. Respondents with a negative test result for *Trichomonas vaginalis* were mostly because they had already undergone treatment. Distribution of CSW respondents based on a questionnaire according to treatment by taking antibiotics, shows that only 30% of CSWs who have taken antibiotics have ever taken antibiotics and 70% have never taken drugs. According to a previous study conducted by Wanda (2017), from 20 urine samples of sex workers in the RRI Surakarta area, no *Trichomonas vaginalis* parasite was found, which means that all respondents were negative, because they had undergone treatment because there were routine checks every 2 weeks by the local health center. Another study conducted by (Ayu, 2017) also stated the same thing that of 30 samples of CSWs in the localization of Sunan Kuning Semarang the majority were negative for *Trichomonas vaginalis* because CSWs had undergone treatment and had local health and laboratory examinations every 6 months, and every 2 months. Once a month, a sexually transmitted infection (STI) is checked. In addition, according to research conducted by (Erna Tristiyanti, 2021) although people with Trichomoniasis have undergone treatment, they can be reinfected, and patients who are undergoing treatment are advised not to have sexual intercourse during treatment before being declared cured. Therefore, both partners must undergo examination and treatment simultaneously to stop the transmission.

Distribution of CSW respondents based on questionnaires according to clinical symptom complaints showed that CSWs who experienced itchy vaginal discharge were 66% and those who did not experience 34%. CSWs who experienced yellowish/greenish vaginal discharge were 47% and those who did not experienced 53%. CSWs who experience smelly/foamy vaginal discharge are 77% and those who don't are 23%. CSWs who experienced vaginal injuries were 47% and those who did not experienced 53%. There were 60% of sex workers who experienced pelvic/lower abdominal pain and 40% who did not. According to the (Frieden et al., 2015), cases of trichomoniasis sufferers rarely show symptoms so that sufferers are rarely aware and most are not treated.

Conclusion

Trichomonas vaginalis was found in urine samples of commercial sex workers who were examined at the PKBI rose clinic in Bandung.

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