



Original Research Article

USING CONVENTIONAL METHODS TO DETERMINE THE LEVEL OF TOILET SEAT CONTAMINATION BY *TRICHOMONAS VAGINALIS* IN THE UNIVERSITY OF EASTERN AFRICA, BARATON, KENYAWilly K Kemboi¹, Jackie Grear² and Ramesh F^{1*}¹Department of Biological Sciences, University of Eastern Africa, Baraton, P.O. Box 2500, Eldoret-30100, Kenya²Department of Medical Laboratory sciences, University of Eastern Africa, Baraton, P.O. Box 2500, Eldoret-30100, Kenya

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Abstract: This study pursued to investigate the possibility of transmission of sexually transmitted disease-Trichomoniasis, via a toilet seat. The disease is caused by a single cell protozoan *Trichomonas vaginalis*. For quite some time, acquisition of Trichomoniasis is believed to be through sexual intercourse and little evidence is via sharing the bathroom tools. A number of investigations have been done and published on the possible relationship that infection with Trichomoniasis escalates the infected susceptibility to HIV/AIDS. In this study, women toilets seats were examined in University of Eastern Africa, Baraton using; wet mount and Gram stain method. The data collected and examined showed that there is statistically significant evidence that trophozoites deposited by infected women on the toilet seats in University of Eastern Africa, Baraton, can cause an infection to health users ($P < 0.05$).

Key Words: Toilet Seat, Trichomonads, Trophozoites, Toilet seat Contamination.

INTRODUCTION

Trichomonas vaginalis is a sexually transmitted parasitic protozoan that lives in the female lower reproductive tract and the male urethra. It causes the sexually transmitted infection trichomoniasis; a disease infecting both males and females. Though both males and females are infected, more cases are reported among females, who are more likely to present with symptomatic infection than males. In females, vaginitis is the most common manifestation of the infection (Chinyere et al., 2010). Trichomoniasis is often asymptomatic in men, however more than half of the infected women manifest vaginitis, cervicitis, urethritis, and irritation with frothy malodorous discharge. *T. vaginalis* can lead to urogenital tract infection (Rezaeian et al., 2009) and may play a critical and under recognized role in amplifying human immune deficiency virus (HIV) transmission. In some circumstances, it may have a major impact on the epidemic dynamics of HIV infection and the acquired immune deficiency syndrome (AIDS) (Uneke et al., 2007).

In the present investigation, an attempt has been made to find out the role of the toilet seat in transmission of *Trichomonas vaginalis*. The study was conducted on contaminated toilet plates as a non-sexual means of transmission of *Trichomonas vaginalis*. Examinations of the samples were done for the presence of infectious trophozoites to ascertain the possibility of spread of the parasite to the health users.

MATERIALS AND METHODS

Study Site

The University of Eastern Africa Baraton is situated in Western Kenya. It is found in Nandi County, off Kapsabet Eldoret highway nine kilometers.

Research Design

In the institution, 30 toilet Seats were examined for five days each week for eight weeks. Each toilet seat was examined using, direct wet mount examination, Grams stain. Each toilet bowl was examined for the presence of *Trichomonas* trophozoites once per day for five days (Monday to Friday) each week for a period of 8 weeks. Samples were acquired from each toilet plate rim. The swab was obtained specifically from the rim where urine hit during micturition and precisely where ladies sit during defecation.

In this study, an unused toilet seat kept as a control medium. Samples collected from this seat were examined using the same three methods and the time period stated for the other toilet seats above.

Population and Sampling Technique

In the present study 30 toilet seats were chosen; 8 from Humanities building, 2 from Room 15 building, which is a toilet sink and not a seat mainly used by employees and 20 from the Ladies dormitory. This represents 30% of the total toilet seats installed specifically for women in the University. Since there were 55 toilet seats in the ladies dormitory all the seats were assigned numbers. The numbers were placed in a box, and the box shaken thoroughly. The first 20 numbers to be picked randomly designate the toilets to be examined for the presence of trichomonads on that

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occasion. In addition, the ladies dormitory, every time the samples were collected, not all the students were present in their rooms and therefore not all rooms were open during the sample collection time. Rooms that were open at time of study were considered statistically random and therefore the first 20 rooms following a zigzag manner up and down the three grounds their toilet seats were chosen every day to participate in the study for five days a week for a period of 8 weeks.

Research Instruments

The following instruments were required during this study; Microscope, Microscope Slides, Gloves, cover slips, tray, Gram stain, Sterile swabs, Normal Saline (Physiologic Saline), Camera, Digital Microscope, Flame, Air Drier, Methanol and Culture tubes.

Data Gathering procedures

The samples were collected by use of a sterile swab. It was first dipped into a normal saline solution and then aseptically used to mop precisely on the spot of the toilet seat where ladies sit and the spot 5.5 cm below the rim where urine hit during urination. The swabbing took 30 seconds and it was transferred immediately to its tube and tightly capped. The samples were transported to the research laboratory to be examined for the presence of Trichomonads within 15 minutes. The wet mount examination was done immediately to observe for the presence of motile trichomonads. After wet mount examination the same samples were used for the Gram stain.

Diagnostic Methods-Examination of the Samples

The diagnostic methods that were employed in this study were:

Microscopy: Wet Mount

Biochemical Methods: Grams staining

Microscopy: Wet Mount

This was the key diagnostic procedure for the study because one of the objectives of the study was to be made specifically by employing this method. The fresh samples collected from the 30 toilets from each sites were examined using this method within 15 minutes. The method employs direct observation of the live trophozoites under x 45 or x10 magnification objective lenses. The samples collected were already wetted with normal saline and the wet mount preparation was done by pressing the swab against the microscope slide then the slide was covered with a cover slip and examined under different magnifications of the microscope. The diagnosis of trichomoniasis has traditionally depended on the microscopic observation

of motile protozoa from vaginal or cervical samples like from the toilet seats and from urethral or prostatic secretions. Donné first described this technique in 1836. *T. vaginalis* can be differentiated based on its characteristic jerky movements. On occasion, flagellar movement can also be noted. The sensitivity of this test varies from 38% to 82% and is dependent on the inoculum size. Equally well, the need for the specimen to remain moist and the experience of the viewer are important. The size of the trichomonad is approximately the same as that of a lymphocyte (10 µm to 20 µm) or a small neutrophil; when not motile, a trichomonad can be difficult to differentiate from the nucleus of a vaginal epithelial cell. Motility is very dependent on the temperature of the specimen. At room temperature in phosphate-buffered saline, the organism will remain alive for more than 6 hours; nevertheless, the motility of the organisms becomes significantly attenuated. This wet mount examination is evidently the most cost-effective diagnostic test, but the lack of sensitivity contributes to the under diagnoses of the disease. Because viable organisms are required, delay in transport and evaporation of moisture from the specimen reduces motility and, consequently, diagnostic sensitivity (Crucitti et al., 2003).

To perform a wet mount, a toilet seat swab was collected from Room 15, Humanities and Ladies Dorm. A positive result required visualization of the characteristic irregular motility of the Trichomonas parasitic organism. While the wet mount test has the benefit of providing a rapid result at the point of care and is inexpensive, the specimen required detection the characteristic motility of the organisms while they are still viable (Nye, 2009).

Bio Chemical Method - Grams staining

Microorganisms can be classified according to their Gram-staining reaction. Gram-positive organisms have thicker and denser peptidoglycan layers in their cell walls. Iodine penetrates the cell wall in these organisms and alters the blue dye to inhibit its diffusion through the cell wall during decolourisation. Gram-positive organisms must have an intact cell wall to produce a positive reaction. Gram-negative cells, which do not retain the methyl/crystal violet, are stained by a counterstain. Neutral red, safranin or carbolfuchsin may be used as the counter stain (Cheesbrough, 1999).

Procedure

A smear was prepared and was heated gently to fix. After which it was flooded with 0.5% methyl crystal violet and left for 30 seconds. The slides were tilted then, poured on sufficient 1% Lugol's iodine to wash away the stain, covered with fresh iodine and allowed acting for 30 seconds. The slide was again

tilted and washed off the iodine with 95% ethanol until color ceased to run out of the smear. The slide was then rinsed with water. After the rinse 0.1% counterstain neutral red was poured, and left to act for about 2 minutes. The slide was then washed with water and blot dried. It was ready for examination under microscope. The *Trichomonas vaginalis* is Gram negative and always stain pink- red with a leaf shape appearance (Cheesbrough, 1999). The samples from different collection sites were stained and examined under different magnification lenses.

Statistical Treatment of the Data

SPSS Data Analysis software version 20 was used to analyze the acquired data and the interpretation was made basing on the output of software. This study had a control and toilet seats in use therefore independent T test was appropriate for this study. The T test is used to find out if there is statistically significant difference in the number of trophozoites between the control and used toilet seats. This investigation was based on previous statistically significant data indicating that it may be possible for one to acquire Trichomoniasis on a toilet seat.

RESULTS

Tables showing average number of trophozoites for a period of 8 weeks in Humanities ladies toilet seats in University of Eastern Africa, Baraton. Samples were stained with Gram stain

Table 1: Average number of trophozoites in the humanities ladies toilet seats

Toilet No.	Day 1	Day 2	Day 3	Day 4	Day 5
	Troph No.	Troph No.	Troph No.	Troph No.	Troph No.
1.	1	0	2	0	0
2.	1	0	0	0	1
3.	1	0	0	2	0
4.	1	1	1	0	6
5.	0	1	0	0	0
6.	1	0	1	1	1
7.	0	2	0	0	0
8.	0	0	2	1	1

Table 2: Average number of trophozoites in the ladies Dormitory toilet seats

Toilet No.	Day 1	Day 2	Day 3	Day 4	Day 5
	Troph No.	Troph No.	Troph No.	Troph No.	Troph No.
1.	0	1	0	1	1
2.	1	0	0	1	0
3.	0	1	2	0	1
4.	0	0	0	2	2
5.	0	0	0	0	0
6.	1	2	1	0	0
7.	1	1	0	0	0
8.	0	1	0	0	0
9.	0	2	1	3	1
10.	0	0	0	0	0

11.	0	0	0	0	0
12.	2	2	0	0	1
13.	1	0	0	1	1
14.	0	0	1	2	1
15.	2	2	0	2	1
16.	0	2	2	1	0
17.	0	2	0	0	0
18.	0	0	2	1	0
19.	1	0	2	1	2
20.	0	0	1	1	1

Table 3: Average number of trophozoites in Room 15 ladies toilet seats

Toilet No.	Day 1	Day 2	Day 3	Day 4	Day 5
	Troph No.	Troph No.	Troph No.	Troph No.	Troph No.
1.	0	3	1	1	2
2.	0	1	0	0	1

Table 4: The average number of Trichomonas trophozoites seen in a control toilet seat

Sample collection day	Diagnostic method	
	Grams stain (No. Trophs)	Wet mount No. Trophs
1.	0	0
2.	0	0
3.	0	0

Key: Trophs = means Trophozoites

The following are the pictures of the *Trichomonas vaginalis* obtained from Humanities building, ladies dormitory and Room 15 stained with Grams stain, viewed under a microscope in University of Eastern Africa

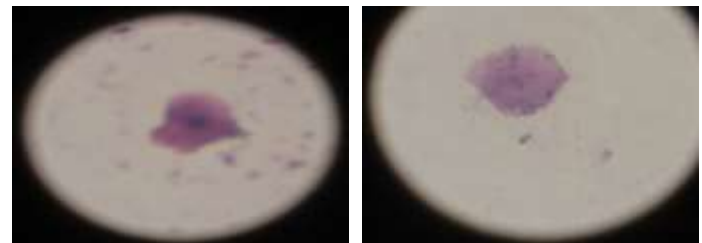


Figure 1: *Trichomonas vaginalis* from samples collected from ladies Dorm toilet seats (Mg x400) Stained with Grams stain.

Statistical Analysis of Data Humanities Building

Table 5: Paired Samples Test, Grams staining

Toilet seats	Mean	Std. Deviation	Sig. (2-tailed)
Unused seats	-.62500	.51755	.011
Used seats			

Ladies Dormitory

Table 6: Paired Samples T Test, Gram stained

Toilet seats	Mean	Std. Deviation	Sig. (2-tailed)
Unused seats	-.80000	.89443	.001
Used seats			

DISCUSSION

The spread of the Trichomoniasis via toilet seat

Toilet seats have been suspected to spread some diseases due to its prone to contaminate. Among the diseases that have been on alert that may be spread via contaminated seats, include *Trichomonas vaginalis*. This study focused on finding out if infected humans can contaminate the toilet seat and spread unquestionably the protozoan to the healthy users when they use a common seat.

The findings from this study showed clearly that the possibility of spread of *Trichomonas vaginalis* low in University of Eastern Africa, Baraton Ladies Dormitory and Humanities building. Though the parasite was not found alive during examination, several conditions may render it immotile for some time and when good conditions prevail for instance user sitting on the toilet seat and being exposed to immotile trophozoite may reactivate it again causing a toilet seat acquired infection.

The center of disease control (CDC), CDC (2012) concluded that the parasite is known to live in wet areas of the genitals, such as the urethra or vagina. While it is most commonly passed from one person to the next during sex, it can sometimes be spread through genital contact with wet towels, wet toilet seats or wet clothing. Trich does not cause any lasting damage for adults but it can cause pregnant women to have premature or low birth-weight babies.

This study differs with CDC (2012) findings and Krieger (2010). In his study Krieger used thermal baths to determine the life span of *Trichomonas* and concluded that it can survive on the toilet seat for more than 3 hours. According to the current study, the thermal baths environment does not be similar to toilet seat environment. The two conditions differ and significantly affect the life span of the parasite as evident in this study by lack of motile trophozoites.

Metabolically active parasites are necessary for lysis of erythrocytes (to cause an infection). Inhibitors greatly reduced erythrocyte lysis, which suggests that a lytic or factor involved in hemolysis. Hemolysis *in vitro* is greatest at the normal vaginal pH of 4.5, suggesting that this parasite characteristic occurs within the vaginal microenvironment (Adegba, 2008). The immotile parasite is metabolically inactive and plays no role in increasing new infections via the toilet seat according to the present study.

This study agrees with above study that it was not possible in UEAB, due to the fact that *T. vaginalis* was not active and hence may not infect health users.

It statistically evident (See the table of Paired Sample t Tests) that the level of contamination on the toilet seats meets the threshold to cause and infection when a health user comes into contact when the trophozoites are still viable (motile). The high number of toilet seat reduces the frequency of visit and consequently reduces the incidences of new infection. If the population of UEAB will increase, the probability of the infection increases due the fact that high population number increases the frequency of the toilet seat visit and as well the chances of meeting a viable Trichomonads.

CONCLUSION

In conclusion, University of Eastern Africa, Baraton women toilet seats are contaminated with *Trichomonas vaginalis*. The number of parasites recorded is statistically significant to cause the infection when still viable. All the parasites that were seen and recorded were not alive and therefore could not cause an infection and as well increase the susceptibility to HIV to women in University of Eastern Africa, Baraton. In a state where one toilet is visited frequently, UEAB toilets are potentially plausible to spread the parasite. Women in University of Eastern Africa, Baraton may be susceptible to HIV/AIDS because of the presence of the Trichomonads on the toilet seats.

There is no enough data to prove statistically that in UEAB, toilet seat contamination in Humanities ladies toilet seats and Ladies dormitory toilet seats can spread the *Trichomonas vaginalis* to health users because none of the parasite was motile during the time of the study.

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