Socio-demographic, Obstetric Characteristics and Molecular Confirmation of *Trichomonas vaginalis* Detected from Pregnant Women Attending Kawo General Hospital, Kaduna, Nigeria

Dikwa K.B[1].; Maryam A.S.[2],; Dibal D.M[3],; Omalu I.C.J[4] and Muhammed Muazu[5]

[1,2,3]Depatrment of Biological Sciences,
Nigerian Defence Academy,
Kaduna, Nigeria

[4]Department of Animal Biology,
Federal University of Technology,
Minna, Nigeria

[5]Pharmacology Laboratory, College of Health Sciences,
Kogi State University,
Anyigba, Nigeria.

Email: kbdikwa@nda.edu.ng

Abstract

Trichomoniasis is a widespread Sexually Transmitted Infections (STIs) that can have detrimental consequences, including infection of the skin, endometrium, Bartholin glands, and fallopian tubes and ovaries (the adnexa of the uterus). This study was conducted to assess the prevalence of Trichomonas vaginalis (T. vaginalis) and T. vaginalis from vaginal swabs of pregnant patients attending Kawo general hospital in Kaduna state. A total of 250 high vaginal swab samples were randomly taken from consenting pregnant women attending antenatal. Structured questionnaires were used to collect participant demographic and medical data. Samples were analysed by wet mount preparation and T. vaginalis positive samples were confirmed using Polymerase Chains Reaction (PCR). Out of the 250 pregnant women that were examined, 24 (9.6%) were positive for T. vaginalis infection. T. vaginalis was detected more in age group 20-25 (9.4%), and among women of third trimester stage (11.4%). T. vaginalis infection was significantly associated with age, gravidation, education and occupation. Among the 24 pregnant women that tested positive for T. vaginalis infection, only 2 (8.0%) were without symptoms associated with vaginal trichomoniasis, while the remaining 22 (92.0%) presented symptoms such as vaginal odour (20%), itching and discomfort (32%) and greenish yellow vaginal discharge (92%). Molecular analysis confirmed 1 positive case out of the 5 samples randomly analysed. Trichomoniasis transmission to newborn infants could be avoided with better personal cleanliness, screening, and treatment for pregnant women who are affected.

Keywords: Socio-demographic, Obstetric, Molecular confirmation, Trichomonas vaginalis, Pregnant women

---

INTRODUCTION

Trichomonas vaginalis is an extracellular single-cell flagellated protozoan parasite, causative agent of Trichomoniasis and the most prevalent and curable human pathogenic protozoan infection that can sexually be transmitted (Kim *et al.*, 2016). It can also be transmitted to newborns while passing via an infected birth canal (Schwebke and Burgess, 2004). The disease is typically asymptomatic, with about one-third of asymptomatic women developing symptoms within six months of infection (Kissinger, 2015). Trichomoniasis present symptoms that includes vaginitis, vulvitis, frothy, yellow-green mucus discharge, purulent, foul-smelling vaginal discharge accompanied by itching, burning, dysuria, dyspareunia (Garber, 2005), infections of the epidermis, endometrium, Bartholin glands and ovaries (the adnexa of the uterus) are symptoms of Trichomoniasis that exposes pregnant women to the risk of other infections and complications following abortion or hysterectomy, infertility, and increased propensity for neoplastic change in cervical tissues (Chinyere *et al.*, 2010). Other symptoms in women typically include vulvovaginal pain, and/or irritation, preterm membrane rupturing, premature labour, and low birth weight (Jahic *et al.*, 2013). In men, prostatitis, nongonococcal urethritis, balanoposthisis, epididymitis, prostitis, and infertility (Cudmore *et al.*, 2004). Lower genitourinary tract symptoms may also develop in males (Uneke *et al.*, 2006).

An estimated 180 million new infections of *T. vaginalis* was reported as the most common nonviral sexually transmitted pathogen in the world (Schwebke *et al.*, 2011), with available evidence indicating that *T. vaginalis* may have a significant and underappreciated role in amplifying HIV transmission and, in certain circumstances, may have a significant impact on the epidemic dynamics of HIV infection and acquired immunodeficiency syndrome (AIDS) in sub-Saharan Africa (Uneke *et al.*, 2007). About 90% of infection in women were from middle-class and lower-class families, while only 10% were from upper-class families (Kaur *et al.*, 2008). The frequency of vaginal Trichomoniasis has been increasing, because the rising prevalence rates in the risk factors for contracting STDs such as HIV and hepatitis B viruses, poverty, low socioeconomic status, a lack of education, high-risk sexual behaviour, incarceration (Rezaeian *et al.*, 2009).

Both men and women with Trichomoniasis have been reported to frequently harbour concurrent infection with other urethral pathogens such *Neisseria gonorrhoeae* and/or *Chlamydia trachomatis*, as untreated infection can continue up to 5 years (Leon *et al.*, 2009). *T. vaginalis* is extremely common and under diagnosed, resulting to increase in HIV susceptibility and transmission (Mabaso and Abbai, 2021). *Colpitis macularis*, or the "strawberry cervix," may be discovered during a colposcopic examination (Dey *et al.*, 2013). Culture methods have been regarded as the most effective way to detect *T. vaginalis* but requires live organisms in comparison to molecular diagnostics that now uses nucleic acid amplification due to the limitations of culture methods and the inadequate sensitivity of sample preparation (Shipitsyna *et al.*, 2012). A PCR targeting *T. vaginalis'* 18S rRNA-DNA genes has enabled the detection of the organism in samples (Mayta *et al.*, 2000). The infection can effectively be treated by the available drugs despite reported worries about rising levels of medication resistance (McClelland *et al.*, 2007; Mavedzenge *et al.*, 2010). In order to prevent re-infection, it is crucial to treat the male partners of infected women (Cudmore *et al.*, 2004). With the increasing prevalence of Trichomoniasis, this study therefore aims to determine the prevalence of *T. vaginalis* and confirm the parasite from vaginal specimen of pregnant women attending Kawo general hospital using PCR.

MATERIALS AND METHODS

Study Area

The study was carried out in Kawo General Hospital located in Kawo, Kaduna North Local Government Area situated within Kaduna metropolis, Kaduna state. Kaduna north is located within latitudes 10^0 35' North and Longitudes 7^0 25' East. It is bordered by Igabi Local Government Area to the South, West, and Southeast, by Kaduna South, Chikun, Kajuru and Kauru Local Government Areas to the Northeast. It has an estimated area of 72 km² and density of 5, 883.1 in h./km². With an estimated population of 423,580 as of 2006 (NPC, 2006; Nipost, 2009) Its wards are Kawo, Unguwan Kanawa/Hayin Banki, Unguwan Dosa, Badarawa/Malali, Unguwan Rimi, Unguwan Shanu/Abakpa, Doka/Gabasawa, Unguwan sarki, Unguwan gaji, Mai burji, Shaba, and Unguwan liman.

Ethical Approval

Ethical clearance was obtained from Kaduna State Ministry of Health before the commencement of this study. The purpose of the study was clearly explained to the study subjects thereby obtaining their participatory consent.

Inclusion and Exclusion Criteria

Consenting pregnant women seeking antenatal care in their current pregnancy at the Kawo General Hospital Kaduna were included while non-consenting antenatal subjects were excluded.

Sample Size Determination

Sample size was calculated according to the formula described by Naing *et al*. (2006), using previous prevalence of 19.2% reported by Solomon *et al*. (2015)

$n = Z^2 p \ (1-P)/d^2$

Where

n = minimum sample size

z = confidence interval at 95%

p = Prevalence in previous study (prevalence of Zaria) = 19.2 % (0.192)

d = precision (acceptable margin of errors = 0.05)

Therefore, $n = (1.96)^2 \ (0.192) \ (1-0.192) / 0.05^2$

$n = 3.8416 \ (0.192) \ (1-0.192) / 0.0025$

$n = 0.5959704576 / 0.0025$

$n = 238.38818304$

The sample size was approximated to 239 so as to cover up for error due to attrition.

Sample Collection

Questionnaires were administered to patients who were attending prenatal clinic for the first time during their current pregnancy in order to acquire their sociodemographic and reproductive profiles. There were two vaginal swabs taken from each individual. To prepare a moist smear, the initial swab was employed. For PCR-based parasite detection, the second swab was collected in a sterile tube with 1 mL of normal saline. According to the procedures outlined by Lawing *et al*. (2000), samples for PCR were treated by freezing for 4 hours. Patients who reported vaginal discharge as well as pruritus, dysuria, and dyspareunia were classified as symptomatic patients (SP). Patients who provided samples but did not express any of the aforementioned symptoms were deemed to be asymptomatic patients (ASP).

Sample Analysis

In 1 mL of normal saline, vaginal swabs were aggressively mixed before being centrifuged at 2000 g for 10 min. The pellet was re-suspended in 1 ml of sterile distilled water after the supernatant was removed, and it was then frozen at -20 °C for subsequent analysis (Inusa *et al.*, 2018).

Wet mount microscopy

A few drops of the pellet suspended in sterile distilled water were mixed with two drops of normal saline to create a smear of the vaginal swab sample, which was then viewed using wet mount microscopy. The presence of *T. vaginalis* was determined through direct observation of the pear-shaped trichomonads with their distinctive jerky parasite movement, and was recognised by the characteristic oval shape, four flagella, and axostyle (Inusa *et al.*, 2018).

Molecular analysis

DNA extraction

The swab samples that yielded positive results in wet mount microscopy were randomly selected across all age groups in order to determine the presence of *T. vaginalis* on such samples using PCR. Following the manufacturer's instructions, the Qiagen DNA extraction kit was used to perform the DNA extraction. All samples and reagents were brought to room temperature prior to the procedure. According to the manufacturer's instructions, 25 and 30 mL of absolute ethanol were used to make Buffer AW1 and Buffer AW2, respectively. 200 µL of vaginal swab suspension and an equivalent amount of Buffer AVL were mixed in a 1.5 mL nuclease-free microcentrifuge tube. Prior to sitting at room temperature for 10 minutes, the mixture was vortexed for 15 seconds. The tubes were centrifuged briefly to clear any drips from the interior of the lid. 400 µL of pure ethanol were added to the sample and vortexed for 15 seconds to mix together it. After mixing, tubes were gently centrifuged once more to remove any remaining drips from the lid. The solution was carefully poured into the QIAamp Mini column (in a 2 mL collection tube) without soaking the rim. The cap was fastened, and the centrifuge was spun at 8000 rpm for one minute. The flowthrough-containing tube was discarded, and the QIAamp Mini column was transferred to a clean 2 mL collecting tube. The QIAamp Mini column was carefully opened, and the remaining solution in the microtube was introduced. A 2 mL collection tube was then closed, and the flowthrough was centrifuged at 8000 rpm for one minute and discarded. The spin column was then filled with 500 µL of Buffer AW1, and the flowthrough was discarded after centrifuging at 8000 rpm for 1 minute. Additionally, 500 l of Buffer AW2 was added, centrifuged for 3 minutes at the maximum speed (14,000 rpm), and the flowthrough was discarded. Without using any reagent, the tubes were centrifuged one more for 3 minutes to spin dry. The previous collection tube containing the filtrate was removed, and a fresh 1.5 mL microcentrifuge tube was used to hold the QIA-amp Mini column (Anneke *et al.*, 2002).

Nested polymerase chain reaction

The actin gene was the target of the nesting PCR. The outer and inner primer sets, which are two sets of primers, performed with various changes (Espinosa *et al.*, 2020).

First round of polymerase chain reaction

The initial amplification of each reaction contained the following components: 8µL of template DNA, 12.5 µL of Top Taq Master Mix, which includes $MgCl_2$, stabilisers, enhancers, and dNTPs (dATP, dGTP, dTTP, and dCTP) in a reaction buffer. 1 litre of each primer combination, 10 pmol, and 1.5 litres of coral load (Qiagen, USA). One atL of nuclease-free water was dispensed to bring the volume to 25 µL. The Polymerase Chain Reaction was cycled in an

Applied Biosystems 9700 thermocycler using the following settings: the first stage consisted of 30 cycles, 30s of denaturation at 95 °C, 30s of annealing at 55 °C, and a 1-min extension at 72 °C (Todd, 2012).

Second round of polymerase chain reaction
For the second round of PCR, the identical reaction mixture with the inner primers was made, the volume was raised to 25µl using nuclease-free water, and 5µl of the amplified product from the first round was used as a template. In this step, the identical denaturation and annealing processes were performed 40 times. The last cycle was followed by a 10-minute final extension at 72 °C. After that, a 100 bp ladder and 10 µl of each amplified product were electrophoretically analysed (Biolabs, UK) (Todd, 2012).
A 1.5% agarose gel with DNA Stain was used to electrophorese each sample. The amplified product was electrophoresed in a 1.5% agarose gel using Tris-Borate EDTA buffer at 80 V. (pH 8.5). After the gel had been coloured with ethidium bromide and examined under ultraviolet light, it was documented using the Bio-Rad XRS gel documentation apparatus. Comparing the sizes of the amplified products to a commercial 100bp weight marker allowed for measurement (Valadkani *et al.*, 2003).

RESULTS AND DISCUSSION
Out of 250 pregnant women examined in this study, 10 pregnant women were in their first trimester of pregnancy (1-3 months) and from these, trophozoites of *T. vaginalis* was not detacted in all (0.00%). On the other hand, 123 pregnant women were in their second trimester (4-6 months) and from these trophozoite of *T. vaginalis* was detected in 14 (11.4%) pregnant women. Likewise, 117 pregnant women were in their third trimester (7-9 months) and from these *T. vaginalis* was detected in 10 (8.5%) pregnant women. From the result obtained, there is a significant difference ($P<0.05$) between the gravida and the number of infected cases (Table 1). This therefore means that there is a significant association between gravida and infection and gravida are dependent on the level of infection.

Table 1: Prevalence of *T. vaginalis* According to Gravida

Gravida	Number Examined	Number Positive (%)	Number Negative (%)
1st Trimester	10	0 (0.0)	10
2nd Trimester	117	10 (8.5)	107
3rd Trimester	123	14 (11.4)	109
Total	**250**	**24 (9.6)**	**226**

Out of the 250 vaginal swabs from pregnant women attending Kawo General Hospital examined, 24 (9.6%) were positive for *T. vaginalis.* Out of the total number examined, 106 women were within the age of 20-25years, 84 within 26-30years, 47 within 31-35 years, 11 within 36-40 years and 2 were of age 40 years and above. *Trichomonas vaginalis* was detected in 10 (9.4%) of women within 20-25years, 7 (8.3%) of those within 26-30years, 5 (10.6%) within 31-35 years, and 2 (18.2%) within 36-40 years, *Trichomonas vaginalis* was not detected in age group 40 years and above 0 (0.0%). From the result obtained, there is a significant difference ($P<0.05$) between the ages observed and the number of infected cases (Table 2). This therefore means that there is a significant association between ages and infection and age is dependent on infection.

Table 2: Prevalence of *Trichomonas vaginalis* According to Age

Age groups (years)	Number examined	Number positive (%)	Number negative
20 - 25	106	10 (9.4)	96
26 - 30	84	7 (8.3)	77
31 - 35	47	5 (10.6)	42
36 - 40	11	2 (18.2)	9
> 40yrs	2	0 (0.00)	2
Total	**250**	**24 (9.6)**	**226**

The prevalence of *Trichomonas vaginalis* based on socio-demographic and obstetric characteristics is presented in Table 3, Out of 250 pregnant women examined, based on educational status, 100 did not attend any form of formal education, from these group of pregnant women, *T. vaginalis* was detected in 13 (13.0%). On the other hand, 91 pregnant women had informal education and out of these, *T. vaginalis* was detected in 7 (7.7%) of the pregnant women. Similarly, 7 pregnant women had primary education and out of these, *T. vaginalis* was detected in 14.3%. Likewise 42 pregnant women had secondary education and out of these, *T. vaginalis* was detected in 7.2% of the pregnant women. Also 10 pregnant women had tertiary education and out of these, *T. vaginalis* was not detected in this category of pregnant women.

Based on occupation, 124 pregnant women were neither working, doing any business nor studying, hence were termed housewives, from these group of pregnant women, *T. vaginalis* was detected in 8.9% of the pregnant women. On the other hand, 7 pregnant women were civil servants and out of these, *T. vaginalis* was detected in 14.3% of the pregnant women. Similarly, 11 pregnant women were students and out of these, *T. vaginalis* was detected in 18.2%. Also, 108 pregnant women were business women and out of these, *T. vaginalis* was detected in 9.3%. Based on gravidity, 78 pregnant women were primigravida and out of these, *T. vaginalis* was detected in 9.0% of the pregnant women. Likewise, 172 pregnant women were multigravida and out of these, *T. vaginalis* was detected in 9.9%. From the results obtained, there is a significant difference (P<0.05) between the educational status and the number of infected cases.

Thus, it can be concluded that there is a noteworthy correlation between educational attainment and infection, with educational attainment being contingent upon infection level. Additionally, a noteworthy distinction exists between the number of infection and occupation. Which means that, the intensity of infection in the less educated respondents is more when compared to the educated respondents. Also, the intensity of infection among the various occupation varies accordingly. Which means that, there is a significant association between occupation and infection as the level of infection varies among the different occupations. Similarly, there is significant difference between gravidation and infection (P<0.05). Which also means that the level of infection and gravidation varies (Table 3).

Table 3: Socio-Demographic and Obstetric Characteristics of Patients with *T. vaginalis* Infection

Characteristics	Number Examined	Number Positive (%)	Number Negative (%)
Educational status			
None	100	13 (13.00)	87 (87.00)
Informal	91	7 (7.69)	84 (92.31)
Primary	7	1 (14.28)	6 (85.72)
Secondary	42	3 (7.14)	39 (92.86)
Tertiary	10	0 (0.00)	10 (100.00)
Occupation			
Housewife	124	11 (8.87)	113 (91.13)
Civil servant	7	1 (14.29)	6 (85.72)
Student	11	2 (18.18)	9 (81.82)
Business	108	10 (9.26)	98 (90.74)
Gravidation			
Primigravida	78	7 (8.98)	71 (91.03)
Multigravida	172	17 (9.88)	155 (90.12)

Out of 250 pregnant women involved in this study, 6 pregnant women had yellowish discharge and out of these, trophozoites of *T. vaginalis* were detected in 1 (16.7%) of them based on wet mount preparation. Likewise 244 pregnant women had white discharge and out of these, trophozoites of *T. vaginalis* were detected in 23 (9.4%) of them based on wet mount preparation. Also none among the pregnant women had colorless discharge. From the results obtained, there is a significant difference (P<0.05) between colour of discharge observed and the number of infected cases. This therefore means that there is a significant association between colour of discharge and infection and colour of discharge are dependent on the level of infection (Table 4).

Table 4: Distribution of *T. vaginalis* in Relation to the Characteristic Appearance of Vaginal Discharge

| Colour of Discharge	Number Examined	Number Positive (%)	Number Negative (%)
Yellowish colour	6	1 (16.67)	5 (83.33)
White colour	244	23 (9.43)	221 (90.57)
Colourless	0	0 (0.00)	0 (0.00)
Total	**250**	**24 (9.60)**	**226 (90.40)**

Out of 250 pregnant women involved in this study, 94 pregnant women had discharge only and from these, trophozoites of *T. vaginalis* were detected in 3 (3.20%) of them. On the other hand, 56 pregnant women had discharge and itching and out of these, trophozoites of *T. vaginalis* were detected in 4 (7.2%) of the pregnant women. Similarly, 71 pregnant women had discharge and dysuria out of which *T. vaginalis* were detected in 5 (7.1%) of the pregnant women. Also, 29 pregnant women had discharge, itching and dysuria out of which trophozoites of *T. vaginalis* were detected in 12 (41.4%) of them (Table 5). From the results obtained, there is a significant difference (P<0.05) between the clinical symptoms observed and the number of infected cases.

Table 5: Distribution of *T. vaginalis* Infection Based on Clinical Symptoms

Clinical Symptoms	Number Examined	Number Positive (%)	Number Negative (%)
Discharge only	94	3 (3.19)	91 (96.81)
Discharge and itching	56	4 (7.14)	52 (92.86)
Discharge and dysuria	71	5 (7.04)	66 (92.96)
Discharge, itching and dysuria	29	12 (41.37)	17 (58.62)
Total	**250**	**24 (9.60)**	**226 (90.40)**

Molecular Confirmation of Wet Mount Sample

Out of the 24 samples that were positive for *T. vaginalis* using wet microscopy, 5 samples were randomly selected and subjected to confirmation via PCR. Results show that only 1 sample was positive at band size 1100-bp as shown in plate I.
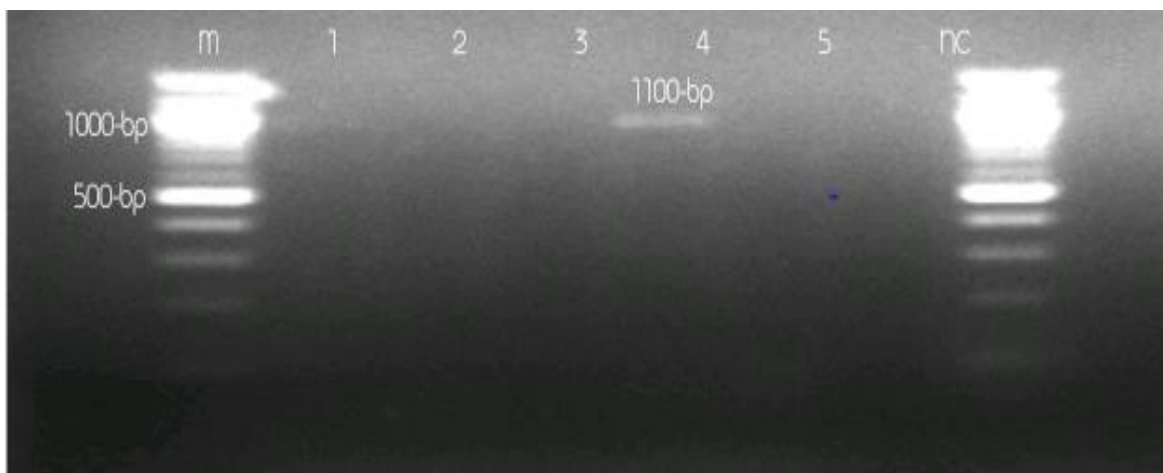
Plate I: Electrophorogram of Amplified PCR products: Lame M molecular ladder (100 bp), lane 1 to lane 5 samples, lane 4 positive sample (1100 bp).

The 9.6% prevalence of *Trichomonas vaginalis* infection in this study is quite high, indicating that nearly one in ten pregnant women are at risk of *T. vaginalis* infection. This is consistent with the prevalence of 10.99% reported by Mairiga *et al.* (2011), and lower than the reports according to Klebanoff *et al.* (2001), indicating a prevalence 7.6% in the US. Donbraye *et al.* (2010) also reported a lower prevalence of *Trichomonas vaginalis* infection of 6.0% in Ibadan, and Abioye *et al.* (2014) observed a prevalence of 7.5% in Nassarawa State.

However, the higher prevalence of *Trichomonas vaginalis* infection recorded by Uneke *et al.* (2007) was 24.4% in Ebonyi, Jatau *et al.* (2006) also reported a prevalence of 18.66 in Zaria, Kaduna State, and Damen *et al.* (2014) claimed a frequency of 16% in Plateau State. These variations could be explained by the different sociocultural lifestyles of the pregnant women studied and by the pregnant women's general awareness of good hygienic habits.

The significantly high prevalence of *Trichomonas vaginalis* infection seen in this study may be attributable to a decline in immunity brought on by ageing, the stress of pregnancy, illiteracy, increased demands placed on the developing foetus as well as the women's reluctance and lack of interest in practising good hygiene.

In comparison to pregnant women with educational backgrounds, more than half of *T. vaginalis*-infected women had no formal education, suggesting that formal education significantly affects the prevalence of *T. vaginalis* infection recorded in this study. This result is consistent with that of Sutton *et al.* (2007), who reported that women with lower literacy

levels had a higher prevalence of *T. vaginalis* than women with higher literacy levels. However, these results diverge from those of Maufi *et al*. (2016), who reported that *T. vaginalis* infection was more common in women with higher literacy backgrounds and less common in those with lower literacy backgrounds.

In this study, the highest prevalence of 11.4% regarding *T. vaginalis* infection was discovered in pregnant women who were in their third trimester of pregnancy. This finding is consistent with those of Mairiga *et al*. (2011), who recorded a higher prevalence of *T. vaginalis* infection at 21.1% among pregnant women in their third trimester. Uneke *et al.* (2005), similarly found a higher prevalence of 8.2% in their third trimester. This high prevalence may be caused by an increase in the demands placed on the developing foetus, which may reduce the mother's immune capacity and enable infection to flourish Damen *et al.* (2014).

Conclusion

There was a significant association between clinical symptoms of Trichomoniasis, gravidation, ages and infection in this study. Clinical symptoms, gravidation and ages were equally dependent on the level of infection. Diagnosis of Tichomoniasis using nucleic acid amplification is more sophisticated than microscopy and culture methods, associated with several limitations

Conflict of Interest

No competing interest exist

REFERENCES

Abdurehman, E., Zeleke, M. and Ahmed, Z. (2013). *Trichomonas vaginalis* Infection among Pregnant Women in Jimma University Specialized Hospital, Southwest Ethiopia, *International Scholarly Research Notices*. doi.org/10.5402/2013/485439.

Abioye, J.O.K., Ihuma, J.O., Abdullahi, D.K., Kamoh, N., Awari, Y.S., Gemma, K. and Babatunde, S.K. (2014) *Trichomonas vaginalis* among Pregnant Women Attending New Nyanya General Hospital, Nasarawa State, Nigeria. *International Journal of Science and Nature*. 5: 437-439.

Aboyeji, A.P. and Nwabuisi, C. (2003). Prevalence of Sexually Transmitted Diseases among Pregnant Women in Ilorin, Nigeria. *Journal of Obstetric Gynaecology* 23:637-9.

Anneke, V., Marcel, P., Caroline, J. and Harold, V. (2002). Qiagen DNA Extraction Kits for Sample Preparation for *Legionella* PCR Are Not Suitable for Diagnostic Purposes. J*ournal of Clinical Microbiology*. 40(3): 1126. doi: 10.1128/JCM.40.3.1126.2002.

Chen, P., Yang, L., Bai, R., Hung, Y.T., (2006). Bakery Waste Treatment. In: Wang, L. K.; Hung, Y.-T.; Lo, H. H.; Yapijakis, C., Handbook of industrial and hazardous wastes treatment, Second edition, *Marcel Dekker, Inc*.

Chinyere, M.E., Sabinus, I.J., Chinedu, C.N., Okoro, N.A., and Chinwe, O.J. (2010). "Prevalence of *Trichomonas vaginalis* among Pregnant Women in Abakaliki, Ebonyi state," *International Journal of Current Research*, 11: 11–15.

Coleman, J.S., Gaydos, C.A., Witter, F. (2013). *Trichomonas vaginalis* Vaginitis in Obstetrics and Gynecology Practice: New Concepts and Controversies. *Obstetric Gynecology Survey*, 68:43-50.

Cudmore, S.L., Delgaty, K.L., Hayward-McClelland, S.F., Petrin, D.P., Garber, G.E. (2004). Treatment of Infections Caused by Metronidazole-Resistant *Trichomonas vaginalis*. *Clinical Microbiology Review*. 17(4):783–793.

Damen, S., Janssen, M. J., Huisman, M., Ruijssenaars, W. A., and Schuengel, C. (2014). Stimulating intersubjective communication in an adult with deafblindness: a single-

case experiment. *Journal of Deaf Studies and Deaf Education.* 19(3): 366–384. doi:10.1093/deafed/enu006.

Dey, S., Rouf, A., Myrthong, L., Suchiang, B., Modak, G., (2013). A Scanning Electron Microscopic Study on Distortion in the Scale of Common Carp, *Cyprinus carpio* Inhabiting a Polluted Lake (Umiam) in North East India. *Journal of Advanced Microscopic Research.* 8: 246-251.

Donbraye-Emmanuel, O.B., Donbraye, E. and I.O. Okonko, I.O. (2010). Detection and prevalence of Candida among pregnant women in Ibadan Nigeria, *World Applied Sciences Journal.* 10 (9): 986-991.

Espinosa, E., Paly, E., and Barre, F.-X. (2020). High-Resolution Whole-Genome Analysis of Sister-Chromatid Contacts. *Molecular Cell.* 79:857–869.

Garber, G.E. (2005). The Laboratory Diagnosis of *Trichomonas vaginalis.* *Canadian Journal of Infectious Diseases and Medical Microbiology.*16:35-38.

Inusa, S.D., Udechukwu, C.U., Panda, S.M., Garba, F.G. (2018). Prevalence of Trichomoniasis among Pregnant Women Attending Ante-natal Clinic in Bauchi state, Nigeria. *Corresponding Author Research Article GSC Biological and Pharmaceutical Sciences,* 02(03): 010–016.

Jahic, M., Mulavdic, M., Nurkic, J., Jahic, E., Midhat, N. (2013). Clinical Characteristics of Aerobic Vaginitis and its Association to Vaginal Candidiasis, Trichomonas Vaginitis and Bacterial Vaginosis. *Medical Archive,* (6): 428-30.

Jatau, E.D., Olonitola, S.A., Olayinka, A.T. (2006). Prevalence of *Trichomonas vaginalis* Infection among Women Attending Antenatal Clinics in Zaria, Nigeria. *Annals of African Medicine.* 5(4):178-181.

Kaur, S, Khurana, S, Bagga, R., Wanchu, A., Nancy, M. Trichomoniasis among women in North India: A hospital based study. *Indian Journal of Sexually Transmitted Diseases.* 29:76-81.

Kim, K., Gitlin, L.N., Han, H.R. (2016). Respond. *American Journal of Public Health.* 106(8):e10-1.

Kissinger, P. (2015). *Trichomonas vaginalis*: A review of epidemiologic, clinical and treatment issues. *BMC Infectious Diseases.* 15:307.

Klebanoff, M.A., Carey, J.C., Hauth, J.C., Hillier, S.L., Nugent, R.P., Thom, E.A., Ernest, J.M., Heine, R.P., Wapner, R.J., Trout, W., Moawad, A., Leveno, K.J., Miodovnik, M., Sibai, B.M., Van Dorsten, J.P., Dombrowski, M.P., O'Sullivan, M.J., Varner, M., Langer, O., McNellis, D., Roberts, J.M. (2001). National Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units. Failure of metronidazole to prevent preterm delivery among pregnant women with asymptomatic *Trichomonas vaginalis* infection. *North England Journal of Medicine.* 16;345(7): 487-93.

Lawing, L.F., Hedges, S.R., Schwebke, J.R. (2000). Detection of Trichomoniasis in Vaginal and Urine Specimens from Women by Culture and PCR. *Journal of Clinical Microbiology* 38: 3585-3588.

Leon, R.R., Konda, K.A., Bernstein, K.T. (2009). "*Trichomonas vaginalis* Infection and Associated Risk Factors in a Socially Marginalized Female Population in Coastal Peru," *Infectious Diseases in Obstetrics and Gynecology.* 2009(752437):1-6.

Mabaso, N. and Abbai, N.S. (2021). A review on *Trichomonas vaginalis* infections in women from Africa. *South African Journal of Infectious Diseases.* 36(1):254. doi: 10.4102/sajid.v36i1.254. PMID: 34485502; PMCID: PMC8377975.

Mairiga, A.G., Balla, H.J., Ahmad, M.I. (2011). Prevalence of *Trichomonas vaginalis* Infections among Antenatal Clients in Maiduguri Nigeria. *International Journal of Biology and Medical Research.* 2(4):998–1002.

Maufi, A.J., Mazigo, H.D. and Kihunrwa, A. (2016). Prevalence and Factors Associated with *Trichomonas vaginalis* Infection among Pregnant Women Attending Public Antenatal Clinics in Mwanza City, North-Western Tanzania. *Tanzania Journal of Health Research.*, 18, 1-7.

Mavedzenge, S.N., Pol, B.V., Cheng, H., Montgomery, E.T., Blanchard, K., de Bruyn, G., Ramjee, G. and Straten, A.V. (2010). Epidemiological Synergy of *Trichomonas vaginalis* and HIV in Zimbabwean and South African Women. *Sexually Transmitted Diseases.* 37(7):460-6.

Mayta, H., Robert, H. G., Calderon, M.M., Gottlieb, A., Soto, G., Tuero, I., Sixto Sanchez, S., and Aldo, V. (2000). 18S Ribosomal DNA-Based PCR for Diagnosis of *Trichomonas vaginalis*. *Journal of Clinical Microbiology.* 38(7): 2683–2687.

McClelland, R.S., Sangare´, L., Hassan, W.M., Lavreys, L., Mandaliya, K., Kiarie, J., Ndinya-Achola, J., Jaoko, W. and Baeten, J.M. (2007). Infection with *Trichomonas vaginalis* Increases the Risk of HIV-1 Acquisition. *Journal of Infectious Diseases.* 195:698–702.

Naing, L., Winn, T. and Rusli, B.N. (2006). Practical Issues in Calculating the Sample Size for Prevalence Studies. *Medical Statistics. Archives of Orofacial Sciences*, 1: 9-14. https://www.scribd.com/doc/63105077/How-to-Calculate-Sample-Size

NPC. (2006). Report of Nigeria's National Population Commission on the 2006 Census. *Population and Development Review.* 33(1): 206-210.

Ochiai, K. Shin-ichi, H., Sachie, N., Setsuko, N., Naomi, I. and Taiki, U.T. (2014). Low blood cell counts in wild Japanese monkeysafter the Fukushima Daiichi nuclear disaster. *Scientifi reports.* 4, 5793; DOI: 10.1038/srep05793.

Ojuromi, T.O., Oyibo, W.A., Tayo, A.O., Ibidapo, C.A., Fagbenro-Beyioku, A.F., Oladosu, O.O. (2007). Reliance on Microscopy in *Trichomonas vaginalis* Diagnosis and its Prevalence in Females Presenting with Vaginal Discharge in Lagos, Nigeria. *Journal of Infections in Developing Countries* 1:210-3.

Olowe, O., Makanjuola, O., Olowe, R., Adekanle, D. (2014). Prevalence of Vulvovaginal Candidiasis, Trichomoniasis and Bacterial Vaginosis among Pregnant Women Receiving Antenatal Care in Southwestern Nigeria. *European Journal of Microbiology and Immunology.* 4, 193-197.

Post Offices- with Map of LGA. NIPOST. (2009). Archived from the Original on 2009-10-07. Retrieved 2009-10-20.

Rezaeian, M., Vatanshenassan, M., Rezaie, S. (2009). "Prevalence of *Trichomonas vaginalis* using Parasitological Methods in Tehran," *Iranian Journal of Parasitology.* 4(4):43–47.

Schwebke, J.R., Hobbs, M.M., Taylor, S.N., Sena, A.C., Catania, M.G., Weinbaum, B.S., Johnson, A.D., Getman, D.K., Gaydos, C.A. (2011). Molecular testing for *Trichomonas vaginalis* in women: results from a prospective U.S. clinical trial. *Journal of Clinical Microbiology.* 49(12):4106-11. doi: 10.1128/JCM.01291-11.

Schwebke, J.R. and Burgess, D. (2004). Trichomoniasis. *Clinical Microbiology Reviews.* (4):794-803, table of contents. doi: 10.1128/CMR.17.4.794-803.2004. PMID: 15489349; PMCID: PMC523559.

Shipitsyna, E., Zolotoverkhaya, E., Chen, C.Y., Chi, K.H., Grigoryev, A., Savicheva, A., Ballard, R., Domeika, M. and Unemo, M. (2012). Evaluation of Polymerase Chain Reaction Assays for the Diagnosis of *Trichomonas vaginalis* Infection in Russia. *Journal of European Academic Dermatology Venereol* 27: 217-223.

Solomon, D.H., Reed, G.W., Kremer, J.M., Curtis, J.R., Farkouh, M.E., Harrold, L.R., Hochberg, M.C., Tsao, P., Greenberg, J.D. (2015). Disease Activity in Rheumatoid Arthritis and the Risk of Cardiovascular Events. *Arthritis Rheumatology.* 67(6):1449-55.

Sutton, M., Sternberg, M., Koumans, E.H., McQuillan, G., Berman, S., Markowitz, L. (2007). The Prevalence of *Trichomonas vaginalis* Infection among Reproductive-Age Women in the United States, 2001–2004. *Clinical Infectious Diseases*. 45(10):1319–26.

Todd, C. L. (2012). Polymerase Chain Reaction: Basic Protocol Plus Troubleshooting and Optimization Strategies. *Journal of visualized experiments*. (63): 3998. doi: 10.3791/3998.

Uneke, C., Alo, J.M.N., Ogbu, O., Ugwuoru, D.C., (2007). "*Trichomonas vaginalis* infection in human immunodeficiency ISRN Infectious Diseases 5 Virus-Seropositive Nigerian Women: The Public Health Significance," *Online Journal of Health and Allied Sciences*. 6(2):3.

Uneke, C.J., Ugwuoru, C.D., Ali, E., Ali, M. (2006). *Trichomonas vaginalis* Infection among Pregnant Women in South-Eastern Nigeria: The Public Health Significance. *International Journal of Gynaecology and Obstetrics* 6:17-21.

Uneke, C.J., Ogbu, O., Inyama, P.U., Anyanwu, G.I., Njoku, M.O. and Idoko, J. (2005). Prevalence of hepatitis B surface antigen among blood donors and human immunodeficiency virus-infected patients in Jos, Nigeria. *Mem Inst Oswaldo Cruz*. 100: 13-16.

Valadkhani, Z., Sharma, S., Harjai, K., Gupta, I., Malla, N. (2003). In Vitro Comparative Kinetics of Adhesive and Haemolytic Potential of *Trichomonas vaginalis* Isolates from Symptomatic and Asymptomatic Females. *Indian Journal of Pathology and Microbiology*. 46(4):693–92003.