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Trichomonas vaginalis infection in southern Ghana: clinical signs associated with the infection

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Trichomonas vaginalis is the causative agent for the most prevalent non-viral sexually transmitted infection (STI) among women of child-bearing age. In Ghana, although the infection is prevalent, there is a dearth of data on the risk factors and symptoms associated with *T. vaginalis* infection. This study was conducted on 492 women visiting gynaecological and STI clinics in the Volta Region (VR) and Greater Accra Region (GAR) in southern Ghana. Wet mount microscopy and polymerase chain reaction (PCR) were used to diagnose *T. vaginalis* infection. Infection prevalence was 13.2% and 18.1% by WMM and PCR, respectively. Diagnosis by PCR was significantly more sensitive (McNemar's test, p=0.0003). The regional prevalence of T. vaginalis infection by PCR was 21.7% in the VR and 12.8% in the GAR. There was a significant difference in prevalence between the two regions (Fisher's exact test, p=0.02). *T. vaginalis* infection was associated with vaginal itch (odds ratio [OR]=1.71, p=0.04) and a history of engaging in oral sex (OR 1.90, p=0.04). A high prevalence of *T. vaginalis* infection was recorded among women visiting gynaecological and STI clinics in southern Ghana. There was no consistent association of infection with any recorded clinical signs and no clear risk factors for infection were identified.

Keywords: rural, southern Ghana, *Trichomonas vaginalis*, urban, women

Introduction

Trichomonas vaginalis is the most common of the non-viral sexually transmitted infections (STIs) worldwide. Globally an estimated 190 million new cases occur annually,[1] mostly in developing countries and socio-economically disadvantaged groups. The infection is associated with a wide range of clinical signs, but none are definitive enough for its determination, with the most common being vaginal discharge, vulva irritation, painful urination, strawberry punctate cervix, yellowish-green frothy vaginal discharge and pain during coitus.[2] *T. vaginalis* infection is also associated with adverse pregnancy and birth outcomes, including miscarriage, pre-term labour, premature membrane rupture, stillbirth and low birthweight.[3,4]

Increasing epidemiological evidence has also indicated the potential role of *T. vaginalis* in both the acquisition[5] and transmission[6] of human immunodeficiency virus (HIV) and has been estimated to have facilitated 6–30% of all new HIV infection cases among African American women in the United States.[7] In sub-Saharan Africa, an estimated 30 million new cases of *T. vaginalis* occur each year.[8] Recent studies in the general population in Zimbabwe and Uganda indicated an up to three times greater HIV infection risk among women infected with *T. vaginalis*.[9] Similarly, an increased risk of HIV among *T. vaginalis*–infected women has been reported in Kenya and Zaire.[4,6]

In Ghana, the national HIV prevalence among pregnant women attending antenatal clinics in 2016 was 2.4%. Among the young population (15–24 y), a proxy for new infections was 1.1%. The prevalence in the 45–49 y age group was highest, at 5.6%, followed by 35–39 y at 3.5% and 15–19 y at 0.6%. The regional HIV prevalence ranged from 2.7% in the Volta and Brong Ahafo Regions to 0.7% in the Northern Region. The prevalence in the Greater Accra Region (GAR) was 2.4%. The national HIV prevalence among STI clients was 5.4%.[10]

Despite the high incidence of *T. vaginalis* worldwide, there is a paucity of data on regional prevalence in most parts of the world. In Ghana, for example, only two previous studies have reported the prevalence of *T. vaginalis* infection, with estimates of 5.4%[11] and 2.7%.[12] Furthermore, prevalence estimates that are available are influenced by the method of diagnosis, age

bracket of study subjects and the sample size.[13,14] The most widely available mode of diagnosis for *T. vaginalis* infection is wet mount microscopy of vaginal fluid specimens, but this technique has a sensitivity as low as 50%.[15] Although wet mount microscopy was considered the preferred technique for diagnosing *T. vaginalis* in the past, a recent study reported a higher sensitivity for polymerase chain reaction (PCR).[16] PCR is now considered the most sensitive method for diagnosing trichomoniasis, according to the Centers for Disease Control 2015 Sexually Transmitted Diseases Treatment Guidelines.[17] Problems with diagnostic sensitivity are compounded by the fact that 50% or more of women infected with *T. vaginalis* show few symptoms or are asymptomatic,[14] thereby resulting in most cases going undetected.

A number of studies have reported risk factors for *T. vaginalis* infection in women in various parts of the world.[18,19] These studies found that age, marital status, education and/or occupation may be associated with an increased risk of *T. vaginalis* infection. There has been no previous investigation of risk factors for *T. vaginalis* infections in Ghana and this presents a major constraint to the management and treatment of the infection in public hospitals and health centres. Presently in Ghana there is no routine screening for this infection, although symptomatic women may be tested by wet mount microscopy. The aims of this study were to estimate the prevalence of *T. vaginalis* in women attending health care clinics in southern Ghana using two different techniques (microscopy and PCR) and assess the clinical signs, sociodemographic and behavioural factors and pregnancy outcomes associated with *T. vaginalis* infection in this region.

Methods

Area and study population

The study was conducted in selected hospitals in the Accra Metropolitan and South Tongu Districts in the Volta Region (VR) of Ghana (Figure 1). The South Tongu District in the VR is predominantly rural, while the Accra Metropolitan District is largely urban, with a higher socio-economic status. The study areas were chosen based on the high number of recurring cases of vaginitis (i.e. high number of reported cases of vaginitis by women who had previously been treated at health facilities and subsequently returned with the same clinical condition) among women visiting the gynaecological and antenatal clinics in these regions. The selected hospitals in these areas serve as the primary and secondary points of care for most residents.

The research was a cross-sectional study and aimed to assess the prevalence of *T. vaginalis* among women visiting the gynaecological and STI clinics of the selected hospitals, as well as assess the clinical signs of infection and the sociodemographic and behavioural factors associated with the infection. The study populations were women living in the South Tongu District in the VR and the Accra Metropolitan District in the GAR, those visiting the South Tongu District Hospital and Comboni Hospital and those visiting the Mamprobi polyclinic and Amasaman Hospital. Inclusion criteria were sexually active women ≥16 y of age, living within the selected communities and visiting the STI and gynaecological clinics of the selected health facilities between January 2016 and April 2016. These women visited these clinics for different reasons, ranging from routine check-ups to diagnosis of observed clinical signs. Women with recent use of antibiotics (within the last month) and those using antibiotics at the time of the study were excluded from the study. In addition, women living outside the selected communities but visiting the clinics in the selected hospitals were excluded from the study. All recruited participants confirmed their willingness to voluntarily participate in the study by signing an informed consent form. Males were not included in this study because of the difficulty in obtaining urethral samples. Additionally, the male counterparts of the women in the study did not accompany the women during their hospital visits, except in rare cases when they were also required to be present since they were involved in the medical procedure.

Ethical approval for the study was granted by the Ethics Committee of Ghana Health Service/Ministry of Health (GHS-ERC 02/11/15) and the Research Ethics and Integrity Committee of Murdoch University (protocol number 2015/164).

Collection of epidemiological data

Structured questionnaires were administered to characterize the socio-epidemiological profile of the study participants. Information was obtained on clinical signs and sociodemographic and behavioural variables (Table 1). Behavioural and demographic variables for this study were selected based on published literature on potential risk factors. Other factors assessed in this study, but not previously regarded in the literature as potential risk factors, were selected based on reported cases of non-sexual transmission of *T. vaginalis*.[20] The HIV status of participants was not included because of the difficulty in obtaining data from all the participants.

Laboratory analysis

Two vaginal secretion swab samples were taken from each participant using sterile non-absorbent cotton swabs. Swabs for wet preparation microscopy were inoculated directly into 0.9% normal saline and transported to the laboratory within 1 h after collection for examination by trained technicians who were staff of the hospital. The results (positive or negative for *T. vaginalis*) were documented and delivered to patients via the nurses for counselling and treatment of infected women. The second swab was analysed using PCR at the State Agriculture and Biotechnology Centre, Murdoch University, Western Australia, Australia, with the tryptophanase (P1)[a] primer and protocol described by Cornelius et al.[21] Following all PCR analyses, subject identifications for the microscopy and PCR results were matched and a list of participants with positive PCR results was submitted to the respective hospitals for follow-up treatment. All participants with positive wet mount microscopy or PCR results were administered a single-dose 2 g metronidazole tablet. This aspect of the work was managed by the nurses and clinicians at the respective hospitals.

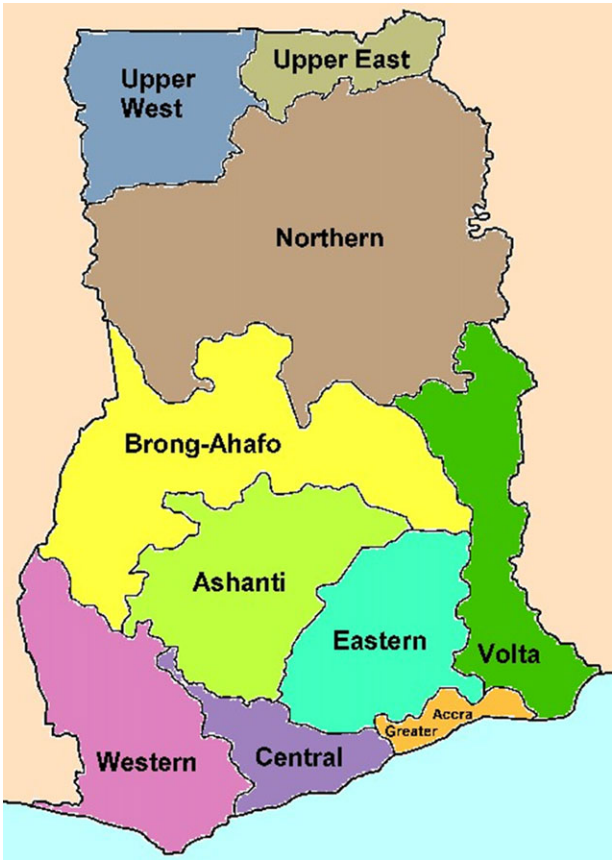

Figure 1. Map of Ghana showing the regions.

Data analysis

All analyses were performed using the R statistical package (R Foundation for Statistical Computing, Vienna, Austria, 2013). Infection prevalence (with 95% confidence intervals calculated assuming a binomial distribution) was estimated from both microscopy and PCR data and the proportion of infected women was compared between diagnostic methods using McNemar's test of agreement.

The relationship between infection with *T. vaginalis* as determined by PCR (the more sensitive diagnostic technique; see Results) and epidemiological variables was investigated separately for clinical signs and sociodemographic/behavioural variables associated with infection. First, for each set of variables the association with infection status was examined by univariable tests (Fisher's exact, χ^2 or logistic regression) and an odds ratio (OR) was calculated. Because there were differences between regions in sociodemographic variables (see Results), these analyses were conducted separately by region as well as for data combined over both regions. For the clinical signs and sociodemographic/behavioural data sets, those variables showing at least a moderate relationship with infection status ($p<0.3$) were retained for a generalized linear model (GLM) analysis with a binomial response variable (*T. vaginalis* infection present or absent) and a logit link function. The region was included as a variable in all GLM analyses. Prior to GLM analysis, correlations among predictor variables were examined by Spearman's ρ, and for any variables that were strongly correlated ($\rho>0.6$), only one was retained for analysis.

The significance of each predictor variable in the GLM was examined by a likelihood ratio test. In addition, we used a multimodel inference approach to determine the relative importance of predictor variables.[22] A set of all possible models was generated from the full GLM using the R package MuMIn.[23] Models were then ranked by the Akaike information criterion, corrected for small sample size (AICc), and model averaging was performed using MuMIn across all models within three AICc values of the best model. The importance of each variable was determined by summing Akaike likelihood weights across all models within the top-ranked set in which the variable occurred, providing the selection probability that a given variable will appear in the AIC best model.[22]

Results

Prevalence of *T. vaginalis* in women from southern Ghana

A total of 492 women were enrolled in the present study. Of these, 290 (58.9%) were from the VR and 202 (41.1%) were from the GAR. The mean age of the participants was 28.5±8.0 y for the VR and 29.8±9.3 y for the GAR. Of the study subjects from the VR, 76.3% (213/279) had <12 y of education (below senior high school education), while 23.7% (66/279) have had >21 y of education (senior high school and beyond). In the GAR, 67.8% (137/202) and 37.2% (65/202) of the subjects had an education level <12 y and ≥12 y, respectively. Among the VR subjects, 63.1% (176/279) were married and 36.9% (103/279) were unmarried. Among the study subjects from the GAR, 25.3% (51/202) were married and 74.8% (151/202) were unmarried.

The overall prevalence of *T. vaginalis* infection was 13.2% (65/492; 95% confidence interval [CI] 9.5 to 17.6) by wet mount microscopy and 18.1% (89/492; 95% CI 12.2 to 21.1) by PCR. There was a significant difference in the positivity rate between the two techniques (McNemar's test, $\chi^2=13$, $p=0.003$). All samples that tested positive by wet mount microscopy were also positive by PCR, while an additional 13 samples that were negative by microscopy were positive by PCR.

The regional prevalence of *T. vaginalis* infection by PCR was 21.7% (62/290; 95% CI 17.1 to 26.9) for the VR and 12.8% (26/202; 95% CI 8.6 to 18.3) for the GAR. There was a significant difference in prevalence between the two regions (Fisher's exact test, $p=0.02$).

Association with epidemiological variables

Eleven subjects who provided inadequate sociodemographic data were excluded from the analyses, leaving 481 (78 positive and 403 negative) samples. Univariable analyses showed no significant association between any of the presenting clinical signs and the presence of *T. vaginalis* when data from different regions were analysed combined or separately, except for discharge colour (OR 2.50, $p=0.04$) in the GAR and discharge consistency (OR 2.18, $p=0.01$) in the VR (Tables 2 and 3). GLM analysis of those predictor variables with $p<0.30$ showed a residual deviance of 414.2 on 475 degrees of freedom,

Table 1. Socio-epidemiological variables collected in the questionnaire (N=492)

Variable	Type	Values
Clinical presentation		
Vaginal discharge	Categorical	Yes/no
Abnormal vaginal discharge	Categorical	Yes/no
Vaginal sores	Categorical	Yes/no
Vaginal itch	Categorical	Yes/no
Painful urination	Categorical	Yes/no
Vulva redness	Categorical	Yes/no
Strawberry cervix	Categorical	Yes/no
Discharge colour	Categorical	Clear/yellowish-green
Discharge consistency	Categorical	Clumpy/cheesy
Discharge frequency	Categorical	Normal/moderate/profuse
Discharge odour	Categorical	Normal/abnormal
Pregnancy outcome history		
Miscarriage	Categorical	Yes/no
Still birth	Categorical	Yes/no
Socio-economic/behavioural		
Age (years), mean±SD	Continuous	28.9±9.1
Education level	Continuous	<High school/high school/tertiary
Marital status	Categorical	Yes/no
Occupation	Categorical	Student/trader/housewife
Region of residence	Categorical	Urban/rural
Pregnancy history	Categorical	Yes/no
Sore	Categorical	Orthodox/herbal/douching
Discharge	Categorical	Orthodox/herbal/douching
Cleaning pattern after using the toilet	Categorical	Back–front/front–back
Cleaning material after using the toilet	Categorical	Toilet tissue, cloth, paper (other than toilet tissue)
Age at first sex (years), mean±SD	Continuous	18.8±2.8
Oral sex practice	Categorical	Yes/no
Saliva lubrication during sexual intercourse	Categorical	Yes/no
Multiple sexual partners	Categorical	Yes/no

suggesting that no correction was necessary for overdispersion. The only significant effect was for vaginal itch (Table 4). Vaginal itch was also ranked highest in variable importance, occurring in 62% of the top eight AICc models; the only other variables to occur in the top set of models were discharge colour, discharge consistency and region (Table 4). Of the 89 infected participants in the study, 33 (37.1%) presented with vaginal itch, 61 (68.5%) presented with some sort of abnormal vaginal discharge, 45 (50.6%) with frothy malodorous discharge and 22 (24.7%) with yellowish-green discharge. *T. vaginalis* infection was not associated with a prior history of miscarriage (Tables 2 and 3).

Univariable analyses found no significant associations between any of the sociodemographic and behavioural variables and the presence of *T. vaginalis* when data were analysed from both regions combined (Table 2). When regions were analysed separately, there was a significant effect of education level in the GAR, where higher levels of infection associated with lower levels of education (Table 3). There was also a significant effect of the age at first sex in both regions, however, the direction of the effect was opposite in the GAR compared with the VR. There was a strong correlation between treatment modes for vaginal sores and vaginal discharge, so only the vaginal sore treatment mode was included in the GLM analysis. The full GLM for those predictor variables with $p<0.30$ in the univariate analyses had a residual deviance of 410.5 on 478 degrees of freedom, again indicating that the data were not overdispersed. The only variable with a significant effect was oral sex (i.e. mouth to vagina); people with a documented history of engaging in oral sex were more likely to be positive for *T. vaginalis* (Table 5). This was also ranked highest in variable importance (69%), with four other variables also occurring in the top-ranked set of models: cleaning material after toilet use (61%), with using pieces of dirty cloth as a cleaning material associated with a greater likelihood of infection; education level (52%), with a lower level of education associated with a greater likelihood of infection; treatment mode (41%), with douching associated with a greater likelihood of infection; and region (29%), with a greater likelihood of infection in the VR (Table 3).

Table 2. Odds ratios and significance of association of each epidemiological variable with *T. vaginalis* infection

Variable	Frequency	*T. vaginalis* positive (%)	Odds ratio	p-Value
Clinical signs				
Vulva redness			1.46	0.40
Yes	48	10 (20.6)		
No	433	68 (15.7)		
Painful urination			1.02	1.00
Yes	116	19 (16.4)		
No	365	59 (16.2)		
Present vaginal discharge			1.17	0.65
Yes	381	60 (15.8)		
No	100	18 (18.0)		
Strawberry cervix			1.61	0.23
Yes	53	12 (22.6)		
No	428	66 (15.4)		
Vaginal sores			1.35	0.38
Yes	113	15 (13.3)		
No	368	63 (17.1)		
Vaginal itch			1.71	0.04*
Yes	238	30 (12.6)		
No	243	48 (19.8)		
Discharge colour			1.64	0.08
Whitish	371	54 (14.6)		
Yellowish-green	110	24 (21.8)		
Vaginal discharge odour			1.36	0.27
Normal	234	33 (14.1)		
Frothy	247	45 (18.2)		
Discharge consistency			1.52	0.10
Clumpy	205	40 (19.5)		
Cheesy	276	38 (13.8)		
Discharge frequency			1.67	0.10
Copious	131	15 (11.5)		
Normal	350	63 (18.0)		
Pregnancy outcome history				
Miscarriage			1.12	0.69
Yes	121	21 (17.4)		
No	360	57 (15.8)		
Socio-economic/behavioural variables				
Age			1.07	0.86
≤21 y	70	12 (17.1)		
≥21 y	411	66 (16.1)		
Education			1.45	0.22
<12 y	348	61 (17.5)		
≥12 y	133	17 (12.8)		
Marital status			1.19	0.54
Single	254	44 (17.3)		
Married	227	34 (14.9)		
Saliva as lubricant			1.15	0.73
Yes	75	11 (14.7)		
No	406	67 (16.5)		
Age at first sex			1.21	0.46
<18 y	252	44 (17.5)		
≥18 y	229	34 (14.9)		

Continued

Table 2. *Continued*

Variable	Frequency	*T. vaginalis* positive (%)	Odds ratio	p-Value
Multiple sex partners**			1.31	0.78
Yes	23	3 (13.0)		
No	458	75 (16.4)		
Pregnancy history			1.55	0.19
Yes	365	64 (17.5)		
No	116	14 (12.1)		
Previous vaginal sore treatment			0.52	0.15
Yes	62	6 (9.7)		
No	419	72 (17.2)		
Occupation			2.52	0.62
Student	49	9 (18.4)		
Trader	188	32 (17.0)		
Housewife	68	14 (20.6)		
Farmer	44	5 (11.4)		
Professional	132	18 (13.6)		
Oral sex			1.90	0.04*
Yes	73	18 (24.7)		
No	408	60 (14.7)		
Vaginal discharge treatment mode			0.90	0.71
Tablets	270	42 (15.6)		
Douching	211	36 (17.1)		
Vaginal sore treatment mode			3.22	0.06
Tablets	432	75 (17.4)		
Douching	49	3 (6.1)		
Toilet cleaning material			1.82	0.12
Tissue paper	386	68 (17.6)		
Piece of cloth	95	10 (10.5)		

*p-Value <0.05 is statistically significant.
Variables included in multivariable analyses shown in bold.
**History of having multiple sexual partners over the last 6 months during the study period.
NB: Eleven participants positive for *T. vaginalis* with incomplete data were excluded from this analysis. The total sample size and number of patients positive for *T. vaginalis* used in this analysis were 481 and 78, respectively.

Discussion

The overall prevalence of *T. vaginalis* infection in this study was 13.2% and 18.1% by wet mount microscopy and PCR, respectively, with a significant difference in sensitivity between the two techniques. The regional prevalence of *T. vaginalis* infection by PCR was 21.7% for the VR and 12.8% for the GAR, with a significant difference in prevalence between the two regions. Vaginal itch and a history of oral sex were the only variables found among the presenting clinical signs and sociodemographic/behavioural factors to have a strong association with *T. vaginalis* infection in this study.

Prevalence

T. vaginalis infection is the most prevalent non-viral STI worldwide.[24] In spite of this, the regional prevalence of the infection has not yet been established in most parts of the world, including Ghana, most likely due to the absence of national routine screening and management and control programmes as exist for other STIs. In most parts of the world, *T. vaginalis* infection has not been considered a high health risk and has received little or no attention with regard to public health and clinical intervention.[25] However, the high prevalence of *T. vaginalis* infection and the associated severe health sequelae observed in recent times requires a more coordinated national programme, similar to other STIs.

In this study, the prevalence of *T. vaginalis* was higher than reported in the two other studies that have been conducted in Ghana.[11,12] These previous studies sampled from only a single hospital and used serological and wet mount techniques to detect infection. Our study, in contrast, sampled women visiting a number of hospitals and clinics and utilized both wet mount microscopy and PCR for diagnosis. We found PCR to be a more sensitive diagnostic method, a finding that has also been reported in previous studies,[26] although the difference in the

Table 3. Odds ratios and significance of association of each epidemiological variable with *T. vaginalis* infection, stratified by region

Variable	GRA	*T. vaginalis* positive	Odds ratio	p-Value	VR	*T. vaginalis* positive	Odds ratio	p-Value
Clinical signs								
Vulva redness			1.78	0.42			1.20	0.81
Yes	15	3			33	7		
No	187	23			246	45		
Painful urination			1.33	0.64			1.09	0.85
Yes	59	9			57	10		
No	143	17			222	42		
Present vaginal discharge			1.10	0.82			1.69	0.19
Yes	144	19			237	41		
No	58	7			42	11		
Strawberry cervix			1.96	0.33			1.45	0.45
Yes	24	5			29	7		
No	178	21			250	45		
Vaginal sores			1.00	1.00			1.65	0.22
Yes	39	5			74	10		
No	163	21			205	42		
Vaginal itch			1.53	0.40			1.76	0.09
Yes	96	10			139	20		
No	106	16			140	32		
Discharge colour			**2.5**	**0.04**			1.56	0.28
Whitish	132	12			239	42		
Yellowish-green	70	14			40	10		
Vaginal discharge odour			1.08	0.86			0.96	1.00
Normal	87	16			147	27		
Frothy	115	10			132	25		
Discharge consistency			1.08	0.86			**2.18**	**0.01**
Clumpy	121	16			84	24		
Cheesy	81	10			195	28		
Discharge frequency			1.84	0.43			1.89	0.08
Copious	37	3			94	12		
Normal	165	23			185	40		
Pregnancy outcome history								
Miscarriage			1.40	0.69			1.36	0.38
Yes	49	5			72	16		
No	153	21			207	36		
Socio-economic/behavioural variables								
Age			1.08	0.56			1.01	0.86
<21 y	22	3			48	9		
≥21 y	180	23			231	43		
Education			**3.07**	**0.04**			0.91	0.86
<12 y	135	22			213	39		
≥12 y	67	4			66	13		
Marital status			2.00	0.24			1.32	0.43
Single	151	22			103	22		
Married	51	4			176	30		
Age at first sex			**17.62**	**<0.001**			**7.56**	**<0.001**
<18 y	154	6			98	38		
≥18 y	48	20			181	14		
Multiple sexual partners			1.19	1.00			1.39	0.75
No	193	25			265	50		
Yes	9	1			14	2		

Continued

Table 3. *Continued*

Variable	GRA	*T. vaginalis* positive	Odds ratio	p-Value	VR	*T. vaginalis* positive	Odds ratio	p-Value
Pregnancy history			1.86	0.33			1.48	0.38
No	47	4			69	10		
Yes	156	23			210	42		
Previous vaginal sore treatment			**2.98**	0.18			1.22	0.79
Yes	37	2			25	4		
No	165	24			254	48		
Occupation			1.04	1.00			1.20	0.81
Student	16	2			33	7		
Trader	68	10			120	22		
Housewife	20	6			48	8		
Farmer	0	0			44	5		
Professional	98	8			34	10		
Oral sex			1.04	1.00			**3.10**	**0.01**
Yes	38	5			35	13		
No	165	21			244	39		
Vaginal discharge treatment mode			1.53	0.48			0.88	0.76
Tablet	150	21			120	21		
Douching	52	5			159	31		
Vaginal sore treatment mode			4.33	0.21			2.42	0.27
Tablet	175	25			257	50		
Douching	27	1			22	2		
Toilet cleaning material			1.39	0.77			2.21	0.06
Tissue paper	172	23			214	45		
Piece of cloth	30	3			65	7		

*p-Value <0.05 considered statistically significant.
Variables with significant association in bold.

Table 4. Association of clinical signs with *T. vaginalis* infection from GLM analysis and model averaging

Source	Z value	p-Value	Importance
Vaginal itch	**2.19**	**0.03**	**0.62**
Discharge colour	1.36	0.17	0.59
Discharge consistency	0.44	0.01	0.51
Region	0.69	0.48	0.32
Discharge frequency	1.72	0.08	0
Strawberry cervix	1.66	0.10	0
Vulvar redness	0.18	0.86	0

Variables are ranked by relative importance, which is the probability of selection in the AIC best-fit model.
Variables significant in the global model are shown in bold

Table 5. Association of socio-economic/behavioural variables with *T. vaginalis* infection from GLM analysis and model averaging

Source	Z value	p-Value	Importance
Oral sex	**2.12**	**0.03**	**0.69**
Cleaning material after toilet	1.15	0.25	0.61
Education	1.62	0.11	0.52
Treatment mode	1.89	0.06	0.41
Region	0.28	0.78	0.29

Variables are ranked by relative importance, which is the probability if selection in the AIC best-fit model.
Variables significant in the global model are shown in bold.

positivity rate was not large, suggesting that wet mount microscopy could still be a valuable bedside test for detection of *T. vaginalis* in Ghana. Because our study population was drawn from women visiting STI and gynaecological clinics, the prevalence we found is likely to be greater than in the general female population in Ghana, although it is similar to prevalences reported elsewhere in sub-Saharan Africa (see Table 6).

Table 6. Prevalence of *T. vaginalis* in Ghana and other countries in sub-Saharan Africa

Country	City	Study group	Prevalence	Reference
South Africa	Durban	Women in high-risk	14.6	27
South Africa	Mopani	Non-pregnant women	20.0	28
Nigeria	Maiduguri	Pregnant women	10.99	29
Nigeria		Pregnant women	18.7	30
Nigeria	Abeokuta	Pregnant women	10.3	31
Southwest Ethiopia		Pregnant women	4.98	32
Northwest Tanzania	Mwanza	Pregnant women	10.41	19
		High-risk women	19.0	33
Kenya	Nairobi	Pregnant women	40.0	34
Ghana	Accra	Pregnant women	2.7	12
	Kumasi	Pregnant women	5.4	11

Association with clinical signs

We found very little consistency in presenting signs of infection reported by participants in this study. Only vaginal itch showed a significant association with *T. vaginalis* infection, although the colour and consistency of vaginal discharge also featured in a high proportion of the top ranked GLMs. Other studies have also found that clinical manifestation of *T. vaginalis* infection is varied with frothy, malodourous vaginal discharge and strawberry cervix being the most useful predictive signs for clinical diagnosis of infection.[2,12] In this study, despite the variations in clinical signs among the study subjects, vaginal discharge and frothy malodourous discharge were presented in more than half of the cases, with less than a fifth of them presenting with strawberry cervix. This variation in clinical signs could be due to a number of reasons, including genetic polymorphism in the parasite[35] and changes in signs with stages of the infection. For example, development of strawberry cervix is progressive with *T. vaginalis* infection, initially developing as an irritable erythema and then progressing into group of small punctate haemorrhagic spots of strawberry appearance on the vagina close to the mucosa of the cervix.[2,12] The clinical signs associated with *T. vaginalis* infection could also be related to the menstrual cycle, as reported in an earlier study,[36] although this was not assessed in the current study and requires further investigation. It is also possible that clinical signs could be altered with varying incubation periods of the parasite and the presence of other infectious agents.[18,32] Other plausible explanations for the variability in clinical presentations among infected participants in this study could be co-infections (for example, with HIV or other infectious agents) or repeated exposure to *T. vaginalis* from infected male sex partners who are not being treated for *T. vaginalis* infection.

The absence of a positive association between most of the presenting signs and *T. vaginalis* infection is not unexpected considering the diverse aetiology of vaginal discharge but could have serious implications for a syndromic approach to managing infection, potentially resulting in under or over treatment, prolonged infection and increased spread in the population. This suggests the need for routine and structured screening and treatment approach to improve the recovery rate of *T. vaginalis* infection in asymptomatic and symptomatic women visiting health and antenatal clinics in Ghana.

Association with sociodemographic/behavioural variables

The only sociodemographic/behavioural variable significantly associated with *T. vaginalis* infection in the general analysis in this study was a previous history of engaging in oral sex, although there was some evidence of association with a lower level of education, using pieces of dirty cloth as a cleaning material after toilet use and douching as a treatment mode for vaginal discharge.

Because this was a cross-sectional study, the identified associations cannot be interpreted as risk factors for infection. In addition, there was little consistency in any of the associations between different geographic regions (VR and GAR), which casts doubt on any causal interpretations. It is not clear how engaging in oral sex may increase the risk of infection. *T. vaginalis* is believed to be confined to the urogenital tract and primarily transmitted through heterosexual vaginal intercourse, although other forms of transmission cannot be completely excluded. A recent case of *T. vaginalis* causing oropharyngeal trichomoniasis in a male patient with a history of engaging in oro-vaginal sex with an infected female partner has been reported.[37] It is possible that oral sexual activity is correlated with unmeasured, confounding factors, but this is speculative at this stage. Previous studies have found evidence for an increased risk of *T. vaginalis* infection with decreased education level, although the association was not strong.[38,39] The linkage existing between level of education and STI is not clearly understood. It is possible that persons with a lower level of education have limited knowledge of safe sexual hygiene and practices, although there could be other confounding factors such as poverty and multiple sexual partners. The weak association of douching with *T. vaginalis* infection found in this study agrees with other findings.[40]

Although we found no association between infection and age in both the general and regional analyses, an increased risk of STI with aging has previously been reported.[41] The high

prevalence among older age groups (>40 y) may be related to biological and hormonal changes resulting in thinning of the vaginal mucosa wall (which acts as a barrier against infection),[42] increasing the risk of a mucosal tear during sexual activity, thereby increasing the risk of infection. In contrast, other studies have found an increased frequency of infection among younger age groups, which could be attributed to higher sexual activity and poor adherence to safe sex practices.[38]

Conclusions

In conclusion, *T. vaginalis* prevalence was high among women attending STI and gynaecological clinics in selected hospitals in the GAR and VR of Ghana. PCR was significantly more sensitive than wet mount microscopy as a diagnostic tool for detecting *T. vaginalis*. Vaginal itching was significantly associated with infection. A number of sociodemographic/behavioural factors for infection were evaluated, with only a history of engaging in oral sex showing a significant association. This study was hospital based, thus the findings may vary slightly from a community-based study in the same population. Also, infection status and treatment of male partners of the women enrolled in this study were not assessed and we had no data on co-infections, limiting the extent to which we could draw conclusions from the association analyses. Nevertheless, the findings from this study may contribute to and inform a new approach in public health and clinical diagnosis of *T. vaginalis* infection in Ghana. Because of the high prevalence of infection, we recommend adoption of a routine screening program and educational package for *T. vaginalis* infection in Ghana. Anecdotal evidence from the study suggests limited knowledge of the parasite, mode of transmission and health implications.

Authors' contributions: All the authors contributed significantly to the study design, its implementation and analysis and interpretation of the data. DSS conceived the study and carried out the experimental work. DSS, RCAT, AJL and JW designed the study protocol and the proposal review for ethics approval, wrote the manuscript in the draft and review stages, analysed and interpreted the data and had a supervisory role during the study. RCAT, AJL and JW critically revised the manuscript for intellectual content. AH carried out the clinical assessment. RHA and AH played a supervisory role at the study site and contributed to interviewing and sample collection at the study sites. All authors read and approved the final manuscript. DSS, AJL, JW and RCAT are guarantors of the paper.

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