Changing Sexually Transmitted Infection Screening Protocol Will Result in Improved Case Finding for *Trichomonas vaginalis* Among High-Risk Female Populations

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Background: *Trichomonas vaginalis* is a sexually transmitted infection, which is largely underestimated because of ineffective screening protocols and lack of public health attention.

Methods: Two studies were conducted to assess the frequency of missed diagnosis of *T. vaginalis* when using current routine practices for *T. vaginalis* screening in high-risk female populations. The first study compares the rate of positivity detected using wet preparation microscopy to the number of cases found using polymerase chain reaction (PCR) using residual samples from women attending a public health sexually transmitted disease clinic. The second study compares universal to targeted screening of symptomatic women using PCR on vaginal samples from women screened for sexually transmitted disease at a correctional facility.

Results: In the first study, a 5-fold increased incidence of *T. vaginalis* infection was detected when PCR was performed instead of wet mount microscopy in a sample of 222 women screened at a sexually transmitted disease clinic. The second study detected a 5-fold increase in cases among a sample of 471 incarcerated women when universal screening was implemented.

Conclusions: Improving detection of *T. vaginalis* is critical, given that when left untreated, *T. vaginalis* increases susceptibility to coinfections including human immunodeficiency virus. Changing screening protocols to use improved diagnostic tools and applying universal screening resulted in increased case finding for *T. vaginalis* among high-risk women. The prevalence of *T. vaginalis* coupled with its negative impact on health necessitate greater public health attention is needed in order to reduce incidence rates, improve diagnosis, and to better understand this important, yet underestimated, pathogen.

Trichomoniasis is a sexually transmitted infection (STI) caused by the motile protozoan *Trichomonas vaginalis* and is the most prevalent nonviral STI, both in the United States and globally.[1] In the United States, there are an estimated 7.4 million incident cases of *T. vaginalis* each year; far exceeding rates of both *Chlamydia trachomatis* (2.8 million new cases)[2] and *Neisseria gonorrhoeae* (>700,000).[2] In the United States, *T. vaginalis* prevalence rates range from 1.3% to 46.9% in women older than 30, and women of color being disproportionately infected by this infection.[3–5]

Trichomoniasis prevalence rates, both worldwide and in the United States, are thought to be largely underestimated due to a lack of reporting, minimal clinical presentation, and the poor sensitivity of *T. vaginalis* diagnostic testing. Screening for *T. vaginalis* is generally limited to women demonstrating overt symptoms, even though 45% to 65% of patients do not present with typical clinical symptoms.[6,7] Further, when diagnostics are ordered,[8–10] most clinical protocols rely on direct examination by wet mount microscopy. However, wet mount is an insensitive diagnostic method with sensitivity ranging from 35% to 60%[6,11] when compared to more robust methods like the nucleic acid amplification test (NAAT) (sensitivity of 96.7% and specificity of 97.5%).[12–14]

Further, since symptoms may be absent or mild, infected individuals may not seek treatment or limit their sexual activity and therefore are more likely to transmit the infection. In a longitudinal study of the natural history of *T. vaginalis* among urban adolescent women, approximately a quarter of participants (57/245) became infected during a 3-month follow-up period (95% confidence intervals [CI], 19.9%–28.5%) and nearly 32% of women with incident cases experienced multiple infections.[7]

Infection with *T. vaginalis* has been definitively linked to increased risk of human immunodeficiency virus (HIV) acquisition and transmission. In a prospective study of 203 HIV-positive women from 1996 to 1998, Wang et al[15] discovered that treating *T. vaginalis* led to a 4.2-fold reduction in cell-free HIV-1 (3.4–3.05 $\log_{10}$ copies/swab; $P < 0.001$) but not HIV-infected cells, in vaginal fluids. A recent nested case–control study found that infection with *T. vaginalis* increases the likelihood of HIV infection acquisition by 2 to 3 fold.[14] *T. vaginalis* was more prevalent among cases than controls (11.3% vs. 4.5%, $P = 0.002$) and a *T. vaginalis* diagnosis was correlated with HIV seroconversion (adjusted odds ratio, 2.74; 95% CI, 1.25–6.00) demonstrating that *T. vaginalis* significantly impacts HIV acquisition.

In an effort to inform future strategies for *T. vaginalis* control among women at high-risk for HIV, we conducted 2 studies in Indianapolis (Indiana) to determine whether minor changes to screening protocol would result in improved *T. vaginalis* case finding. Study 1 describes the increase in positivity rates among women seeking care at a local public health sexually transmitted disease (STD) clinic when NAAT is used in lieu of wet mount microscopy. Study 2 compares the effect of universal screening for *T. vaginalis* to targeted testing based

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on self-reported symptoms among incarcerated women. Indianapolis was chosen as the study site because it historically had some of the highest rates of STD in the United States[16] and is currently experiencing an increase in cases of syphilis and chlamydia. Given the frequent occurrence of concomitant STD and having a *T. vaginalis* infection increases HIV susceptibility,[8,9,13,14] we wanted to explore methods to improve case finding as an STD control strategy.

METHODS

Study 1

Laboratory records were reviewed to determine the number of female public health STD clinic patients, at least 18 years of age, who were tested for *T. vaginalis* DNA between October and December 2008. Samples were collected using a self-administered vaginal specimen and were tested using a previously described polymerase chain reaction (PCR) that is a modification of the Amplicor CT/NG assay (Roched Molecular, United States).[6] We reviewed the electronic medical records of these patients to determine whether wet preparation microscopy had also been ordered at the time of the patient's clinical visit and whether *T. vaginalis* infection was correctly diagnosed using PCR. We then compared the rate of positivity detected using wet preparation microscopy to the number of cases found when we utilized PCR.

Study 2

Samples were obtained from women incarcerated in a privately operated minimum security facility in Marion County (Indiana) that is a designated STD sentinel surveillance site. For surveillance purposes, self-obtained vaginal swabs from all consenting women arriving in the facility are routinely tested for chlamydia and gonorrhea. PCR for *T. vaginalis* was routinely offered only to those women with symptoms (targeted screening for trichomonas). For this evaluation, we prospectively performed PCR for trichomonas on all vaginal samples routinely collected for chlamydia/gonorrhea testing (universal screening of all women willing to be tested). Due to the sensitivity of working with a detainee population, historic controls were used to compare the number of cases identified by targeted versus universal screening. No alternate *T. vaginalis* diagnostics were performed. The number of cases of *T. vaginalis* identified by universal screening was compared with the number of cases identified during a control period in which only targeted testing was performed. To control for the possibility of rapid shifts in disease prevalence, rates of *C. trachomatis* and *N. gonorrhoeae* were compared across the 2 periods as well.

For both studies, the Indiana University/Clarian Institutional Review Board approved all data collection and analysis procedures.

RESULTS

Study 1

A total of 1674 women were screened for STD at the Bell Flower Clinic between October and December 2008. Based on self-reported symptoms of discharge, dysuria, or pelvic pain, 222 (13.2%) were screened for *T. vaginalis* using both wet preparation microscopy and PCR. Microscopy as the diagnostic tool detected *T. vaginalis* in 6 of 222 (2.7%) patients. PCR as the diagnostic tool detected *T. vaginalis* in 30 of 222 (13.5%) patients. PCR resulted in a 5-fold increase in *T. vaginalis* case finding.

TABLE 1. Comparison of CT, GC, TV Positivity Rates by Study Period

	Historical Period N = 97	Study Period N = 471
Trichomonas vaginalis (TV)	51 (52.5%)	208 (52.6%)
Chlamydia	9 (10.5%)	57 (12.1%)
Gonorrhea	8 (9.1%)	25 (5.3%)

CT indicates *Chlamydia trachomatis*; GC, *Neisseria gonorrhea*.

Study 2

Historical control data were extracted from testing performed in the population of 362 incarcerated females opting to be tested for STD from April to July 2007, immediately preceding our study. Using a targeted screening approach based on self-reported symptoms yielded 97 of 362 women who were screened for *T. vaginalis* testing; 51 of 97 symptomatic women screened were identified as positive for trichomonas DNA (Table 1). However, this approach detected trichomonas in only 14% of the entire female detainee population (51/362).

When *T. vaginalis* testing was performed on all samples (471) collected from August to November 2007, as opposed to only those with self-reported symptoms, 208 cases of *T. vaginalis* were detected. Although the trichomonas positivity rate in the historic control group, 52.6% (95% CI, 42.6, 62.6) was high, as would be suspected when testing a targeted population, the rate observed during universal screening (44.2% [95% CI, 42.6%, 45.7%]) was not significantly lower and over 4 times as many cases were detected.

We also compared positivity rates for gonorrhea and Chlamydia between the 2 time periods in order to rule out any rapid shifts in overall disease rates. During the historical control period, the prevalences of chlamydia and gonorrhea were 10.5% and 9.1%, respectively. The rates were similar during the universal screening period, 57 of 471 (12.1%) and 25 of 471 (5.3%) for chlamydia and gonorrhea, respectively, which suggests that a shift in overall disease prevalence had not occurred between the 2 time periods.

DISCUSSION

We present 2 studies that demonstrate how changing screening protocols, by utilizing improved diagnostic tools and applying universal screening, resulted in increased case finding for *T. vaginalis* among high-risk women. As a result of these studies, the procedures used in Marion County have been changed to include the following: (1) NAAT testing for trichomonas in all wet mount negative women being tested for chlamydia and gonorrhea and (2) inclusion of trichomonas in all orders for chlamydia and gonorrhea testing on samples obtained from the Department of Corrections. Although this study was performed using a laboratory-developed assay (modified PCR), evidence of the performance of other NAAT for detection of trichomonas nucleic acids suggests that the specific method is not critical. As commercially available tests that allow chlamydia, gonorrhea, and trichomonas testing on the same platform become available,[10] these tests should be considered for inclusion in the routine screening process in women at high risk for negative outcomes associated with *T. vaginalis* infection.

Improving detection of *T. vaginalis* is critical, given that when left untreated, *T. vaginalis* can lead to increased susceptibility to coinfections including HIV. Infections are frequently

missed due to ineffective clinic screening protocols including the restriction of screening to women demonstrating overt symptoms and the use of insensitive diagnostic methods. Given the exceptionally low number of women diagnosed with trichomonas in nationally representative studies of prevalence (2.3%–3.1%)[4] targeting services exclusively to symptomatic women will fail to address the need to reduce the burden of disease in high-risk populations.[12] In order to achieve this goal, modified clinic screening protocols are needed.

In our first study, routine clinical diagnosis using wet mount missed 24 of 30 (80%) of *T. vaginalis* cases demonstrating that microscopy is an ineffective diagnostic tool for this pathogen given its low sensitivity compared to NAAT. In our second study, historical controls were used to compare case finding using targeted diagnostics of symptomatic women to universal screening of women in a correctional facility. Universal screening, in a high-prevalence population, resulted in a 4-fold increase in the number of positive cases found. Clearly, because of the asymptomatic nature of this infection, control of this disease is unlikely to be successful when only women with symptoms are tested.

A limitation of these analyses is that PCR results could only be compared to wet mount microscopy results when the test was ordered by the clinician; some patients did not have a wet mount ordered and were therefore not included in our analysis during the first study and microscopy was not available for any women in the correctional setting (second study). This may lead to an incorrect representation of the number of cases undiagnosed, however, this limitation would not account for a 5-fold increase in detection using PCR. Among the female detainee sample, STI screening is an optional service; not all women choose to participate, thus the true prevalence of infection in this population remains unknown. However, this applies to the rates of chlamydia and gonorrhea as well and these were similar among the 2 study periods suggesting that there has not been a dramatic increase in overall prevalence of STI in this population.

Aggressive testing, treatment, and surveillance programs should be instituted due to the burden of *T. vaginalis* cases and the substantial evidence linking *T. vaginalis* to adverse health outcomes, including pelvic inflammatory disease,[17] preterm delivery and low birth weight,[18] and cervical cancer.[19] Most importantly, this pathogen is suspected to have played a major role in the HIV pandemic because it increases viral shedding in HIV-infected individuals and causes inflammation in genital tissues that increase susceptibility in HIV-negative partners.[15,20]

The prevalence of *T. vaginalis* coupled with its negative impact on health makes this STI a significant public health concern. In order to reduce rates of this infection, a call to action is necessary and should include improved diagnostic tools, modifying clinic screening protocols, and addition of this important infection to the list of notifiable diseases should be considered. Bundling *T. vaginalis* testing with gonorrhea and chlamydia may be necessary for detecting this infection in high-prevalence populations who are also at risk for HIV. However, studies of the cost-benefit of *T. vaginalis* control programs are clearly warranted in order to provide justification for the addition of this test to the standard panel of tests offered by STD control programs.

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