Prevalence, Incidence, Natural History, and Response to Treatment of *Trichomonas vaginalis* Infection among Adolescent Women

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(See the editorial commentary by Schwebke, on pages 2036–8.)

**Background.** *Trichomonas vaginalis* infection is a sexually transmitted infection (STI) linked with reproductive health complications. However, few data exist concerning the epidemiologic profile of this pathogen in adolescent women, a group at high risk for other STIs.

**Methods.** Our objective was to describe the prevalence, incidence, natural history, and response to treatment of *T. vaginalis* infection in adolescent women. Women 14–17 years old were followed for up to 27 months. Vaginal swab samples were obtained during quarterly clinic visits and were self-obtained weekly during 12-week diary collection periods. The weekly samples were tested quarterly. Infections were identified by polymerase chain reaction and were treated with 2.0 g of oral metronidazole. Analysis was performed on the subset of participants who returned for at least 1 quarterly clinic visit.

**Results.** *T. vaginalis* infection was identified in 6.0% (16/268) of the participants at enrollment. Overall, 23.2% (57/245) of the participants with at least 3 months of follow-up had at least 1 infection episode; 31.6% (18/57) experienced multiple episodes. Seventy-two incident infection episodes were diagnosed. When treatment was not documented, weekly samples from participants were positive for up to 12 consecutive weeks. After treatment, *T. vaginalis* DNA was undetectable within 2 weeks in all but 3 participants.

**Conclusions.** The incidence of *T. vaginalis* infection is high among adolescent women; untreated infections may last undetected for 3 months or longer. Reinfection is common. Treatment with oral metronidazole is effective, and *T. vaginalis* DNA disappears rapidly after treatment.
PARTICIPANTS, MATERIALS, AND METHODS

Participants. Participants were adolescent women receiving health care at 1 of 3 primary health clinics in Indianapolis, Indiana. These clinics primarily serve lower- and middle-income residents of areas that have high rates of teenage pregnancy and STIs. Clinic patients were eligible for enrollment if they were between 14 and 17 years old, spoke English, and were not pregnant at the time of enrollment. However, enrolled participants who became pregnant were retained in the study cohort. Prior sexual experience was not a requirement for participation.

Study design and procedures. Data were collected as part of a larger longitudinal study (initiated in 1999) of risk and protective factors associated with STIs among young women in middle adolescence. Briefly, the parent study included up to 10 clinical interviews and examinations; these occurred at enrollment and quarterly thereafter. Vaginal swab samples for STI diagnostics were collected during the clinic visits and were run in real time, to provide data for clinical management. In addition, data and swab samples were collected during up to five 12-week diary collection periods during a 27-month study period. During each diary collection period, up to 11 weekly visits (typically at the participants’ homes) were conducted by research staff, to collect diaries and self-obtained vaginal swabs from participants. Diary collection periods were followed by rest periods in which no diary information was collected. Each diary collection period was bracketed by clinic visits for collection of interview and physical-examination data. These visits allowed research personnel to reinforce diary collection procedures, maintain current contact information, and treat any infections identified using the weekly samples. Informed consent was obtained from each participant, and permission was obtained from a parent or legal guardian. This research was approved by the Institutional Review Board of Indiana University–Purdue University Indianapolis/Clarian Health.

At enrollment and each subsequent quarterly clinic visit, face-to-face interviews were used to obtain information on sexual behaviors, including condom use. Vaginal swab samples were obtained by a research nurse practitioner for diagnosis of STIs. These samples were tested in real time, with results available to research staff within 72 h. A prescription for 2.0 g of oral metronidazole was provided to all participants with a T. vaginalis infection; those found to be infected on the basis of the assay. Therefore, a conservative definition of an infection episode was established with respect to the diary collection period, requiring at least 2 positive results. Data from women who attended at least 1 quarterly clinic visit were included in the analysis of T. vaginalis prevalence and incidence. Point prevalence was calculated as the number of infections identified at enrollment divided by the number of participants included in the analysis. Cumulative prevalence was defined as the total number of participants ever infected during the course of the study divided by the number of participants included in the analysis. The first infection identified during follow-up in those participants who were negative at enrollment and infections in participants for whom effective treatment of previous infections was documented were classified as incident episodes. Onset of an infection episode was defined as the collection date of the first positive sample. Duration of an infection episode was defined as...
the interval between the onset of infection and the collection date of the first of at least 2 sequential negative weekly samples. For missing weekly samples, infection status was considered to be negative unless the samples collected immediately before and after the missing sample were positive. DNA shedding was defined as a positive weekly test after documented treatment. Therapy was considered to be effective when weekly samples became negative within 3 weeks of treatment and remained negative for at least 2 weeks.

RESULTS

The average age of the 268 participants enrolled in the parent study was 15.4 years; 233 (86.9%) were black. Sixteen (6.0% [95% confidence interval (CI), 3.2%–8.8%]) received a diagnosis of \textit{T. vaginalis} infection during the enrollment visit. In this cohort, the prevalences at enrollment of \textit{Chlamydia trachomatis} and \textit{Neisseria gonorrhoeae} infection were 10.1% (95% CI, 6.5%–13.7%) and 4.1% (95% CI, 1.7%–6.5%), respectively. Longitudinal analysis was restricted to the participants who had returned for at least 1 quarterly clinic visit after enrollment (245/268). Of the 245 participants with at least 1 quarterly clinic visit, 13 (5.3%) had received a diagnosis of \textit{T. vaginalis} infection at enrollment, whereas a total of 57 (cumulative prevalence, 23.2% [95% CI 17.9%–28.5%]) had at least 1 infection episode at some point during follow-up. Eighteen (31.6%) had multiple infection episodes during the study period, with up to 4 separate episodes in 2 women. The mean ± SD number of infection episodes in the 43 infected women who completed follow-up was 1.6 ± 0.9.

Forty-five (18.4%) participants had at least 1 incident infection episode, with 72 incident episodes occurring overall. Thirty-seven incident episodes were identified using the weekly samples; in 21 of these instances, using the sample obtained during the next scheduled quarterly clinic visit, the participants were not found to be positive. Documentation of treatment during an interim clinic visit was found for 11 of these 21 participants. The mean ± SD time to seeking treatment for these 11 participants was 2.9 ± 2.3 weeks (median, 2 weeks). The remaining 10 participants had no documented treatment, and the reason for the subsequent negative samples is unknown.

For 42 infection episodes, documentation of treatment was available along with subsequent weekly samples to verify treatment efficacy. For all but 3 episodes (7.0%) in 3 participants, \textit{T. vaginalis} DNA was no longer detectable within 2 weeks of the treatment date. One participant remained positive for an additional 8 weeks after treatment, and 2 remained positive for an additional 12 weeks after treatment (until the next scheduled quarterly clinic visit), at which time they were again treated.

Saline wet-mount preparation results were available for 1409 quarterly clinic visits, including 61 of 64 infection episodes that were identified by PCR. Motile trichomonads were observed by microscopy in 31 wet-mount preparations, including 26 (42.6%) of the 61 from clinic visits during which infection episodes were identified by PCR. Five positive wet-mount preparations were not positive by PCR using the sample obtained during that clinic visit. However, 3 of 5 participants who had positive wet-mount preparations but negative PCR results were never positive by PCR during the subsequent 12 weeks of follow-up; this suggests the possibility of false-positive wet-mount preparation results. These 3 participants with positive clinical tests were classified as being uninfected, on the basis of the PCR results. The remaining 2 participants had subsequent positive weekly samples and were classified as being infected. Taken together, these data suggest that the sensitivity of the PCR assay is between 91.8% and 96.7%.

Information on symptoms was available for 54 of the 61 quarterly clinic visits for which wet-mount preparation results were available. Seventeen (54.8%) of the 31 positive wet-mount preparations were from symptomatic participants, all of whom had vaginal discharge. In our study population, abnormal discharge was observed in 29 (53.7%) of 54 participants who were positive for \textit{T. vaginalis} infection by PCR. Thus, negative wet-mount preparations or the absence of vaginal discharge are insufficiently sensitive to reliably rule out \textit{T. vaginalis} infection. Bacterial vaginosis (diagnosed on the basis of the whiff test and the presence of clue cells) was identified in 11.5% of the \textit{T. vaginalis}–infected participants, and yeast infection was diagnosed microscopically in 18.6%.

A variety of patterns of positive and negative test results were observed during follow-up; examples of some typical patterns observed in the participants who were included in the analysis are shown in figure 1. Many of the patterns are easily interpreted, such as test results after successful treatment, test results for an incident infection episode for which interim treatment was sought, and test results for an incident infection episode that persisted until the next quarterly clinic visit (examples 1, 2, and 3, respectively, in figure 1). However, the interpretation of other patterns is more subjective. We interpreted example 4 as successful treatment followed by reinfection, on the basis of information on coital activity contained in the diary. Example 5 could be the result of identical exposures, as seen in example 4, because there was a weekly period with missing data; however, in the absence of any documented coital activity and with only 1 week of documented negative test results, the period was classified as potential treatment failure.

Example 6 was interpreted as a single infection episode, on the basis of the absence of documented treatment and a high level of coital activity during the diary collection period. The occasional negative test results were assumed to be the result of either low organism load or inadequate sample collection. However, there are a number of possible scenarios that could result in a similar pattern. Missing data also hamper the in-
Figure 1. Eight examples of patterns of positive and negative test results over 12 consecutive weeks. Each square represents a weekly self-obtained vaginal swab sample and diary collection unit, with the hatched squares indicating positive test results and the missing squares indicating missed collection weeks. The black arrows indicate quarterly clinic visits, the black triangles indicate treatment with oral metronidazole, and the white hearts indicate coital activity. The patterns were interpreted as follows: (1) successful treatment; (2) incident infection episode for which interim treatment was sought; (3) incident infection episode that persisted until the next quarterly clinic visit; (4) successful treatment followed by possible reinfection, on the basis of information on coital activity; (5) potential treatment failure, on the basis of there being only 1 week of documented negative test results, compounded by no documented coital activity; (6) single infection episode, on the basis of the absence of documented treatment and a high level of coital activity (however, this complicated pattern of positive and negative results, coital events, and missing data could be the result of a number of possible scenarios); (7) undocumented treatment, natural clearance of infection, or an infection level below the limit of sensitivity of the assay; and (8) exposure without infection or false-positive result.

Interpretation of such patterns. Patterns such as example 7 are also subject to multiple interpretations, including undocumented treatment, natural clearance of infection, or an infection level below the limit of sensitivity of the assay.

Example 8 shows a single positive weekly sample. Because an infection episode was defined as at least 2 positive weekly samples, the analysis did not include those participants who had an isolated positive weekly sample. This happened in 7 instances. One participant was treated during the same week, one was treated within 2 weeks, 2 were treated within 4 weeks, and the remaining 3 had no record of treatment. Sample quality may explain why some samples were negative even though the participants sought interim treatment. The 3 samples from participants for whom no treatment was documented could represent false-positive results or the detection of DNA in the absence of active infection that had cleared spontaneously.

The durations of infection episodes were limited by treatment during quarterly clinic visits or during interim clinic visits in all but 10 instances. Eleven participants sought treatment during an interim visit, and 8 of 11 did so within 2 weeks of onset; the remaining 3 participants did not seek treatment until 6–7 weeks after onset. This rate of seeking treatment within 2 weeks (72.7%) is similar to the percentage of infections that were symptomatic when identified during quarterly clinic visits (68.5%).

DISCUSSION

In this longitudinal observational study, T. vaginalis infection was found to be common, occurring in 23.2% of the study participants. The point prevalence at enrollment (6.0%) was in the range of that seen for C. trachomatis (10.1%) and N. gonorrhoeae (4.1%) infection. The prevalences of all of these STIs is particularly high when one considers that not all of the participants were sexually active during the entire study. The participants were not required to be sexually active to be included, and the young women often experienced extended periods of abstinence. As a result of our not controlling for this type of state change, the denominators in our calculations produced underestimations of the actual prevalences of infections. In future analyses, we hope to include behavioral data to control for such exposure variables as coital activity, use of condoms, and partner infection status.

As we have previously documented, reoccurrence of infection also appears to be common in a similar population studied; in that population, >30% of the ever-infected participants experienced >1 T. vaginalis infection episode [8]. In part, the high reinfection rate may reflect a lack of aggressive efforts to identify and treat infected sex partners for this nonreportable infection; however, we were unable to find evidence in clinical trials that would indicate the efficacy of partner treatment in reducing T. vaginalis reinfection rates. Also, it should be remembered that we were unable to distinguish reinfection from persistence or treatment failure in the present study. The difficulty with these types of analyses can be seen in the complexity of the patterns of positive test results shown in figure 1.

Compared with wet-mount preparation microscopy, the standard in most settings, the use of PCR for detection of T. vaginalis DNA offered a significant improvement in the ability to identify infections. As a result of the behavioral nature of the parent study, the number of samples collected was kept to a mini-
mum, and T. vaginalis culture was not performed. Thus, the exact performance characteristics of the T. vaginalis PCR assay cannot be known, given the lack of a diagnostic reference standard. However, similar performance characteristics are observed when the T. vaginalis PCR assay used in the present study is compared with an alternate nucleic acid amplification test that uses primers directed against a different target [15]. The effect of freezing the weekly samples was assumed to be minimal, because of the concordance among the results obtained using the frozen weekly samples obtained during the diary collection periods and the never-frozen samples obtained at the end of these periods during quarterly clinic visits. In 14 of the 16 instances where weekly samples were collected immediately after documented treatment, DNA was detectable for at least 1 week, even though the samples had been frozen for up to 12 weeks. Similarly, of the 20 instances in which weekly samples were collected before a positive sample was obtained during a quarterly clinic visit, there were some number of positive weekly samples for 14, despite freezing. In the other 6 instances, it is possible that the infection was acquired during the week before the quarterly clinic visit.

On the basis of the present data, it appears that women who seek treatment for T. vaginalis infection are likely to do so within 2 weeks of the onset of an infection episode. Thirty-seven infection episodes were first identified by PCR using the weekly samples, and the participants sought interim medical care for less than one-third of them, as documented in our countywide database. This result suggests that infections may be asymptomatic and long in duration and underscores the importance of studies examining the health outcomes of routine screening programs for asymptomatic T. vaginalis infection.

The present data have several limitations that should be considered. First, day-to-day symptoms were not assessed during the diary collection periods. Mild symptoms may have been present during apparently asymptomatic infections, and symptoms may have been misconstrued as normal vaginal discharge. Second, it is possible that we were unable to document all effective treatment that could explain apparent spontaneous resolutions of infections. Third, partner treatment was not verified. Finally, missing data raised questions regarding a variety of behaviors, in addition to leaving the infection status undetermined. In complex patterns of positive and negative test results, missing data points make interpretation uncertain. Furthermore, it is important to emphasize that these data were obtained from a highly motivated cohort of young women who were willing to provide both multiple samples and detailed behavioral data. As such, it is not our intention that the findings for this cohort be generalized to broader populations.

The data presented here indicate that untreated T. vaginalis infection can persist for up to 12 weeks and that individuals can be asymptomatic throughout the duration of an infection. Because of the method we used for determining the onset of an infection episode, the actual average duration of infection may be longer than the results presented here. For example, if a participant was first positive in her third weekly sample, she may actually have acquired the infection 6 days previously. If the infection cleared before the quarterly clinic visit or after treatment, the infection may have actually lasted up to 6 days after the last positive sample was obtained. Clearly, truncating the time results in a conservative estimate of the duration of infection.

In the present study, had we been able to diagnose T. vaginalis infection only on the basis of a combination of wet-mount preparation microscopy and clinical signs, we would have detected only 68.5% of infections. This suggests a somewhat higher frequency of infection than what has been assumed in the literature, especially in the absence of systematic surveillance efforts. The lack of symptoms during a large proportion of infections (31.5%) is an additional cause for concern with respect to the management of sexual health in adolescent women. It appears likely that a large number of infections, lacking both symptoms and clinical signs, remain undetected. Although the current recommendations for STI control include at least annual screening for C. trachomatis and often concurrent testing for N. gonorrhoeae, the potential for T. vaginalis infection is largely ignored, even though it may be more prevalent than chlamydia infection and gonorrhea combined in some populations. As urine and self-obtained vaginal swabs become more widely accepted as source samples for STI screening, use of a self-obtained swab for saline wet-mount preparations in clinical settings may be prudent [16]. The commercial availability of DNA amplification tests and more-sensitive point-of-care tests would be additional useful tools [17].

The present data also demonstrate that treatment with 2.0 g of oral metronidazole appears to be an efficacious regimen with a failure rate of, at most, 7.0%. For 85% of the participants, T. vaginalis DNA shedding was undetectable within 2 weeks of treatment. Given the risk of complications (including adverse outcomes of pregnancy and HIV infection) and the availability of effective treatment, our data suggest that increased attention should be paid to the control of T. vaginalis infection, concurrent with chlamydia infection and gonorrhea control programs.

References
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