The Association between *Trichomonas vaginalis* Infection and Level of Vaginal Lactobacilli, in Nonpregnant Women

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The effect that vaginal lactobacilli have on trichomoniasis is not known. At 3 clinics for treatment of sexually transmitted disease, we recruited 521 female patients with trichomoniasis and 176 control subjects. All participants underwent physical examinations and testing for sexually transmitted infection and completed questionnaires. Low levels of vaginal lactobacilli were associated with trichomoniasis (odds ratio [OR], 2.2 [95% confidence interval [CI], 1.4–3.2]). After adjustment for covariables, this association remained in women with a higher educational level (OR, 4.6 [95% CI, 2.2–9.5]) but not in women with a lower educational level (OR, 1.6 [95% CI, 0.7–3.4]). Vaginal lactobacilli may be associated with trichomoniasis in women with higher levels of education or a related behavioral factor.

Trichomoniasis is the most common curable sexually transmitted infection (STI) worldwide [1]. The prevalence of *Trichomonas vaginalis* infection in women in the United States is 6%–54%, depending on the population studied [2]. Trichomoniasis can lead to health problems, including pelvic inflammatory disease and preterm delivery, and is a risk factor for HIV infection [3]. Until recently, trichomoniasis has received little clinical and research attention, despite its public health importance.

*Lactobacillus* species are the most prevalent organisms in normal human vaginal flora and may protect the vagina against infection [4]. The addition of lactobacilli to in vitro *T. vaginalis* culture slows growth of the protozoa, and *T. vaginalis* phagocytes lactobacilli [5]. However, epidemiologic evidence is inconsistent [6–9], and the association between *T. vaginalis* and lactobacilli is not understood.

We conducted a case-control study to assess the association between level of vaginal lactobacilli and *T. vaginalis* among nonpregnant women. Although this topic has been examined previously, this is the first study of nonpregnant women to have an adequate sample size, use the diagnostic reference standard, and comprehensively evaluate the effect that covariables have on the association between the level of vaginal lactobacilli and *T. vaginalis*. Clarifying this relationship could improve our understanding of *T. vaginalis* and may have implications for the treatment of trichomoniasis.

**Participants, materials, and methods.** We conducted a prospective, case-control study in 3 health-department sexually transmitted disease clinics, located in Durham and Raleigh in North Carolina and Birmingham in Alabama, from November 2001 to July 2003. For every 3 women with positive wet mount–microscopy results for *T. vaginalis* whom we enrolled, we recruited 1 woman with negative wet mount–microscopy results; this case:control ratio was selected as part of a larger study that focused on *T. vaginalis*–infection concordance between infected women and their male partners [10]. Female patients who were ≥18 years old, spoke English, and had not previously been prescreened or enrolled in the study were eligible for prescreening. Women were prescreened on the basis of wet mount–microscopy results and *T. vaginalis* cultures. Prescreened patients with positive wet mount–microscopy results for *T. vaginalis* were recruited immediately and completed questionnaires at enrollment; prescreened patients with negative wet mount–microscopy results for *T. vaginalis* were contacted and asked to return for treatment and/or counseling; during the follow-up visit, they were recruited for the study and completed questionnaires. The unmatched control group was selected from prescreened women whom wet-mount microscopy had found to be uninfected with *T. vaginalis*, but control subjects whom *T. vaginalis* cultures subsequently identified as having trichomoniasis were reclassified as case patients.

Prescreened women who had a history of vaginal sex during...
the preceding 60 days and had not used metronidazole during the 4 weeks before *T. vaginalis* cultures were collected were eligible for enrollment in the study. All enrolled participants provided written informed consent, and the study was approved by the University of North Carolina at Chapel Hill and the University of Alabama at Birmingham Biomedical Institutional Review Boards.

Vaginal swabs for Gram staining and for *T. vaginalis* culturing were obtained from prescreened women. Vaginal wet-mount microscopy and routine testing for *Neisseria gonorrhoeae* were performed. Routine testing for *Chlamydia trachomatis* was also performed, although in 1 of the 3 clinics it was limited to women ≤25 years old. All participants underwent routine physical and bimanual pelvic examinations. Clinical, demographic, and behavioral information was obtained from medical records and a detailed questionnaire.

At each of the 3 clinics, wet-mount microscopy was performed according to routine clinic procedures performed by trained laboratory personnel. *T. vaginalis* culture was performed by use of the InPouch culture system (Biomed). Cultures were read daily for up to 5 days after inoculation. Vaginal smears were sent to the University of Alabama at Birmingham for Gram-stain evaluation and were quantified by use of criteria of Nugent et al. [11]. Bacterial vaginosis was diagnosed on the basis of the Nugent et al. score for the vaginal Gram stain [11]. Testing for *N. gonorrhoeae* was performed by use of culturing or DNA hybridization.

Data were analyzed by use of SAS software (version 8). Women with positive wet-mount–microscopy results or positive *T. vaginalis* culture results were classified as having trichomoniasis. Women with negative wet-mount–microscopy results and negative *T. vaginalis* culture results were defined as not having *T. vaginalis* infection. We conducted descriptive analyses of the study population by examining the frequency distribution, measures of central tendency, and variability for covariates.

The level of vaginal lactobacilli was measured by use of the *Lactobacillus*-species vaginal Gram-stain score based on the categories described by Nugent et al. [11]. Bivariable analyses were performed in parallel, using both dichotomous variables (based on data on the distribution of the vaginal lactobacilli) and 4-level indicator variables for vaginal lactobacilli. Because both coding schemes yielded similar results, we dichotomized the data on vaginal lactobacilli to facilitate interpretation of the study. Women who had ≥30 lactobacillus morphotypes per oil-immersion field (i.e., a *Lactobacillus*-species vaginal Gram-stain score of 4+) were classified as having “high” levels of vaginal lactobacilli, whereas women who had ≤30 lactobacillus morphotypes per oil-immersion field (i.e., a *Lactobacillus*-species vaginal Gram-stain score of 0 to 3+) were classified as having “low” levels of vaginal lactobacilli.

We used bivariable analyses to evaluate both potential confounding and intersubgroup differences in the association between the level of vaginal lactobacilli and *T. vaginalis*. Differences between the subgroups were assessed by use of the Breslow-Day test of homogeneity. Variables that resulted in *P* < .20 were assessed in multivariable analyses of intersubgroup differences. Variables that were associated with both the level of vaginal lactobacilli and *T. vaginalis* were considered to potentially confound the association between them; we evaluated these variables by using a stratified tabular approach [12]. Unconditional multiple logistic regression was used to estimate an adjusted odds ratio (OR) and 95% confidence interval (CI) for the association between the level of vaginal lactobacilli and the presence of trichomoniasis.

We used a backward-elimination model–building strategy. Interaction terms were assessed by comparing the likelihood ratios (LRs) for models with and without the interaction term. Variables resulting in *P* ≤ .10 for an LR were retained in the model. Confounding of the association between the level of vaginal lactobacilli and *T. vaginalis* was assessed by comparing the fully adjusted OR to the adjusted OR after potentially confounding variables had been removed until the cumulative change in the OR of the exposure was >10%.

**Results.** Of the 3836 women prescreened, 790 were diagnosed as having *T. vaginalis* infection. Of these, 540 (68.4%) infected women participated in the study and 521 (65.9%) were included in the present analyses (19 were excluded because of missing data). Of the 203 women negative for *T. vaginalis* recruited as controls for the study, 177 (87.2%) agreed to participate. One uninfected woman was excluded because of missing data.

The median age of participants was 27 years (SD, 9 years), and most (91%) were non-Hispanic black, and have a lower level of education and of household income than did women without *T. vaginalis* (table 1). The proportion of women who reported having douch during the preceding 2 months, and most (78.6%) of those who had done so had used a commercial douche preparation.

Women with *T. vaginalis* tended to have lower levels of vaginal lactobacilli, be non-Hispanic black, and have a lower level of education and of household income than did women without *T. vaginalis* (table 1). The proportion of women who reported having douch during the preceding 2 months and at least once during the preceding week was higher in those with *T. vaginalis* than in those without *T. vaginalis*.

Demographic variables such as non-Hispanic black race (OR, 2.1 [95% CI, 1.2–3.7]), lower educational level (OR, 2.2 [95% CI, 1.5–3.2]), and lower household income (OR, 1.5 [95% CI, 1.0–2.3]) were positively associated with low levels of vaginal lactobacilli.
Table 1. Characteristics of women in the study.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Participants, no. (% of women in category)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case patients</td>
<td>Control subjects</td>
</tr>
<tr>
<td>Level of vaginal lactobacilli (n = 697)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;=30/field</td>
<td>438 (61)</td>
<td>125 (40)</td>
</tr>
<tr>
<td>&gt;30/field</td>
<td>83 (12)</td>
<td>51 (17)</td>
</tr>
<tr>
<td>Age (n = 697)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;=45 years</td>
<td>36 (5)</td>
<td>2 (0)</td>
</tr>
<tr>
<td>40–44 years</td>
<td>65 (9)</td>
<td>8 (3)</td>
</tr>
<tr>
<td>35–39 years</td>
<td>71 (10)</td>
<td>15 (5)</td>
</tr>
<tr>
<td>30–34 years</td>
<td>69 (10)</td>
<td>26 (9)</td>
</tr>
<tr>
<td>25–29 years</td>
<td>90 (13)</td>
<td>41 (14)</td>
</tr>
<tr>
<td>18–24 years</td>
<td>189 (27)</td>
<td>84 (28)</td>
</tr>
<tr>
<td>Race/ethnicity (n = 697)</td>
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<td></td>
</tr>
<tr>
<td>Non-Hispanic black</td>
<td>485 (68)</td>
<td>148 (48)</td>
</tr>
<tr>
<td>Other</td>
<td>36 (5)</td>
<td>28 (9)</td>
</tr>
<tr>
<td>Last year’s household income (n = 648)</td>
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<td></td>
</tr>
<tr>
<td>&lt;$12,000</td>
<td>260 (39)</td>
<td>64 (22)</td>
</tr>
<tr>
<td>&gt;$12,000</td>
<td>222 (33)</td>
<td>102 (34)</td>
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<tr>
<td>Education (n = 697)</td>
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<tr>
<td>High school or less</td>
<td>363 (53)</td>
<td>91 (30)</td>
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<tr>
<td>More than high school</td>
<td>158 (23)</td>
<td>85 (28)</td>
</tr>
<tr>
<td>Marital status (n = 697)</td>
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<td></td>
</tr>
<tr>
<td>Married</td>
<td>45 (6)</td>
<td>19 (6)</td>
</tr>
<tr>
<td>Separated</td>
<td>33 (5)</td>
<td>7 (2)</td>
</tr>
<tr>
<td>Divorced</td>
<td>13 (2)</td>
<td>60 (20)</td>
</tr>
<tr>
<td>Widowed</td>
<td>6 (1)</td>
<td>0 (0)</td>
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<tr>
<td>Single, never married</td>
<td>377 (54)</td>
<td>137 (46)</td>
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<tr>
<td>No. of times patient has sex/average week (n = 674)</td>
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<tr>
<td>&gt;=1</td>
<td>451 (67)</td>
<td>163 (54)</td>
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<tr>
<td>0</td>
<td>52 (8)</td>
<td>8 (3)</td>
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<tr>
<td>No. of sex partners in preceding 2 months (n = 696)</td>
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<td></td>
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<tr>
<td>&gt;=2</td>
<td>177 (26)</td>
<td>42 (14)</td>
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<tr>
<td>0 or 1</td>
<td>343 (51)</td>
<td>134 (45)</td>
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<tr>
<td>Bacterial vaginosis status (n = 697)</td>
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<tr>
<td>Positive</td>
<td>271 (39)</td>
<td>91 (30)</td>
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<tr>
<td>Negative</td>
<td>250 (36)</td>
<td>85 (28)</td>
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<tr>
<td>Coinfection</td>
<td></td>
<td></td>
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<tr>
<td>Neisseria gonorrhoeae (n = 682)</td>
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</tr>
<tr>
<td>Yes</td>
<td>47 (7)</td>
<td>5 (2)</td>
</tr>
<tr>
<td>No</td>
<td>460 (68)</td>
<td>170 (57)</td>
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<tr>
<td>Chlamydia trachomatis (n = 573)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>49 (7)</td>
<td>16 (6)</td>
</tr>
<tr>
<td>No</td>
<td>374 (55)</td>
<td>134 (45)</td>
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<tr>
<td>Previous diagnosis of STI$^b$ (n = 694)</td>
<td></td>
<td></td>
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<tr>
<td>Yes</td>
<td>409 (59)</td>
<td>138 (47)</td>
</tr>
<tr>
<td>No/Don’t know</td>
<td>109 (16)</td>
<td>38 (13)</td>
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<tr>
<td>Oral-contraceptive use$^c$ (n = 693)</td>
<td></td>
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<tr>
<td>Yes</td>
<td>48 (7)</td>
<td>31 (11)</td>
</tr>
<tr>
<td>No</td>
<td>470 (69)</td>
<td>144 (49)</td>
</tr>
</tbody>
</table>

(continued)
Table 1. (Continued.)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Participants, no. (% of women in category)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case patients</td>
<td>Control subjects</td>
</tr>
<tr>
<td>Condom use&lt;sup&gt;c&lt;/sup&gt; (n = 686)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>223</td>
<td>83</td>
</tr>
<tr>
<td>No</td>
<td>293</td>
<td>87</td>
</tr>
<tr>
<td>Douched during preceding 2 months (n = 694)</td>
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<td></td>
</tr>
<tr>
<td>Yes</td>
<td>270</td>
<td>66</td>
</tr>
<tr>
<td>No</td>
<td>249</td>
<td>109</td>
</tr>
<tr>
<td>No. of douches during average week (n = 627)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;1</td>
<td>72</td>
<td>14</td>
</tr>
<tr>
<td>0</td>
<td>389</td>
<td>152</td>
</tr>
<tr>
<td>Usual douching preparation (n = 671)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Commercial preparation&lt;sup&gt;d&lt;/sup&gt;</td>
<td>195</td>
<td>51</td>
</tr>
<tr>
<td>Water and vinegar&lt;sup&gt;d&lt;/sup&gt;</td>
<td>55</td>
<td>12</td>
</tr>
<tr>
<td>No douche during preceding 2 months</td>
<td>249</td>
<td>109</td>
</tr>
</tbody>
</table>

<sup>a</sup> Adjusted for race/ethnicity, age, diagnosis of bacterial vaginosis, coinfection with *Neisseria gonorrhoeae*, condom use, oral-contraceptive use, no. of times patient has sex during an average week, and no. of sex partners during preceding 2 months.

<sup>b</sup> Other than trichomoniasis.

<sup>c</sup> With most recent and/or frequent sex partner.

<sup>d</sup> Among women who had douching during preceding 2 months.

Lactobacilli. Oral-contraceptive use and condom use were inversely associated with low levels of vaginal lactobacilli (OR, 0.4 [95% CI, 0.2–0.6] and 0.6 [95% CI, 0.4–0.8], respectively). Douching during the preceding 2 months was associated with lower levels of vaginal lactobacilli (OR, 1.8 [95% CI, 1.2–2.6]), as was douching more than once during an average week (OR, 2.0 [95% CI, 1.0–3.9]). Neither sexual behavior nor coinfection with either *Neisseria gonorrhoeae* or *Chlamydia trachomatis* was associated with the level of vaginal lactobacilli.

In bivariable analyses, low levels of vaginal lactobacilli were positively associated with *Trichomonas vaginalis* (OR, 2.2 [95% CI, 1.4–3.2]) and remained so after adjustment for age, race, education, concurrent bacterial vaginosis, concurrent gonorrhea, number of sex partners during the preceding 2 months, number of times the woman had sex during an average week, and oral contraceptive use (adjusted OR, 2.8 [95% CI, 1.6–5.0]).

The results of multivariable analyses suggested that the association between the level of vaginal lactobacilli and *T. vaginalis* differs by educational level (*P* = .02 for the LR). Among women with more than a high school education, low levels of vaginal lactobacilli were positively associated with *T. vaginalis* (OR, 3.3 [95% CI, 1.8–5.9]); this association was not observed among women with a high school education or less (OR, 1.1 [95% CI, 0.6–2.0]). Among women with more than a high school education, low levels of vaginal lactobacilli remained positively associated with *T. vaginalis* after adjustment for the same cofactors that had been adjusted for in the bivariable analyses (adjusted OR, 4.6 [95% CI, 2.2–9.5]). Among women

Table 2. Association between vaginal lactobacilli and *Trichomonas vaginalis*.

<table>
<thead>
<tr>
<th>Vaginal lactobacilli</th>
<th>Participants, no. (% of women in category)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case patients</td>
<td>Control subjects</td>
</tr>
<tr>
<td>High school or less (n = 454)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;=30/field</td>
<td>310</td>
<td>77</td>
</tr>
<tr>
<td>&gt;30/field</td>
<td>53</td>
<td>14</td>
</tr>
<tr>
<td>More than high school (n = 243)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;=30/field</td>
<td>128</td>
<td>48</td>
</tr>
<tr>
<td>&gt;30/field</td>
<td>30</td>
<td>37</td>
</tr>
</tbody>
</table>

<sup>a</sup> Adjusted for race/ethnicity, age, diagnosis of bacterial vaginosis, coinfection with *Neisseria gonorrhoeae*, condom use, oral-contraceptive use, no. of times patient has sex during an average week, and no. of sex partners during preceding 2 months.
with a high school education or less, *T. vaginalis* was not independently associated with low levels of lactobacilli after adjustment for potentially confounding variables (adjusted OR, 1.6 [95% CI, 0.7–3.4]). These associations, which are presented in Table 2, were not substantially altered after the final model was adjusted for *C. trachomatis* coinfection and for previous *T. vaginalis* infection.

**Discussion.** Trichomoniasis is the most common curable STI and an apparent contributor to HIV acquisition [1]. The relationship between vaginal lactobacilli and *T. vaginalis* remains unclear, although it could affect important aspects of infection; for example, the presence of abnormal vaginal flora after cervicovaginal conditions and antimicrobial treatment of these conditions may promote relapse of infection [13]. A clearer understanding of the role that lactobacilli play in the natural history of *T. vaginalis* may help refine this therapy and possibly improve the treatment of *T. vaginalis*.

In the present study, lower levels of vaginal lactobacilli were found to be positively associated with prevalent *T. vaginalis*. The association between the level of vaginal lactobacilli and *T. vaginalis* varied between women with different levels of education: among women with a high school education or less, *T. vaginalis* was not independently associated with low levels of vaginal lactobacilli; however, such an association was found among women with more than a high school education, after adjustment for covariables that could potentially confound this relationship.

The reason for the observed education-dependent effect between these groups of women is unclear; given the relative imprecision of the education-specific estimates, this observation may have been due to chance. However, education may also be a proxy for another factor, which we either did not measure or did not measure well. We explored whether, in multivariable models, education could be a proxy for other study variables, such as race, douching, and geographic region; however, an education-correlated difference in adjusted OR was not observed for them, and, furthermore, they were not strongly associated with education, as a proxy would be. The associations between the level of vaginal lactobacilli and *T. vaginalis* when multilevel indicator variables were used for the level of vaginal lactobacilli and educational level were also consistent with those observed when dichotomous coding of these variables was used. The education-correlated difference that was observed is probably due not to educational level itself but to another factor associated with education, a factor that was not captured in this study.

We were unable to explore whether the level of vaginal lactobacilli and *T. vaginalis* infection change over time, and prospective studies are needed to clarify whether low levels of vaginal lactobacilli increase the risk for *T. vaginalis* infection or whether *T. vaginalis* reduces the level of vaginal lactobacilli. Furthermore, vaginal flora are dynamic, and the level of lactobacilli measured in the present study may not reflect the level of lactobacilli present when the participant was infected with *T. vaginalis*; however, a study of vaginal flora has shown that the level of lactobacilli does not significantly change during the menstrual cycle [14].

The results of the present study suggest that lower levels of vaginal lactobacilli are associated with the presence of *T. vaginalis* in women with higher educational levels (or a factor related to higher educational levels) and that this relationship is not explained by other demographic, clinical, or behavioral factors measured in the present study. Elucidation of this relationship may improve our understanding of the pathogenesis, acquisition, and management of trichomoniasis.

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**References**


