The Effect of Vaginal Lactobacilli and Estrogen on Trichomoniasis in Non-Pregnant Women

Michelle Torok

A dissertation submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Epidemiology.

Chapel Hill
2007

Approved by

Advisor: William C. Miller

Reader: Marcia M. Hobbs

Reader: Peter A. Leone

Reader: Pia D. Macdonald

Reader: Arlene C. Sena- Soberano

ABSTRACT

Michelle Torok: The Effect of Vaginal Lactobacilli and Estrogen on Trichomoniasis in Non-Pregnant Women

(Under the direction of William C. Miller)

This research focused on the effect of vaginal lactobacilli and estrogen on trichomoniasis. Vaginal lactobacilli were evaluated by vaginal Gram stain, using criteria described by Nugent [1]. Women with ≥30 large *Lactobacillus* spp. morphotypes per oil immersion field were classified as having higher vaginal lactobacilli levels. Women with ≤30 large *Lactobacillus* spp. morphotypes per oil immersion field were classified as having lower levels. After adjusting for age, race/ethnicity, education, concurrent bacterial vaginosis (BV), concurrent gonorrhea and/or syphilis, number of sex partners in the past 2 months, number of times the woman has sex in an average week, and oral contraceptive (OC) use, low levels of vaginal lactobacilli were positively associated with *Trichomonas vaginalis (Tv)* among women with greater than a high school education (odds ratio [OR]=4.6; 95% confidence intervals [CI], 2.2-9.7). *Trichomonas vaginalis* was not associated with low levels of lactobacilli among women with less than or equal to a high school education in multivariable analysis (OR=1.5; 95% CI, 0.7-3.5).

The association between OC use and *Tv* was also estimated. Women who reported using OC with their most recent and/or frequent sexual partners were categorized as using OC. Women who did not report using OC with their most

recent and/or frequent sexual partner were classified as not using OC. After adjusting for race/ethnicity, highest educational level attained, condom use, concurrent BV, level of vaginal lactobacilli, number of sex partners in the past year and number of times the woman has sex in an average week, OC was moderately protective against *Tv* (OR= 0.6, 95% CI 0.3-1.0).

These data suggest that trichomoniasis may be influenced by level of vaginal lactobacilli and OC use.

ACKNOWLEDGEMENTS

I would like to thank my advisor and dissertation committee chair, Bill Miller, for his even-keeled approach to writing a dissertation. He is also a gifted epidemiologist and teacher, for which I am most grateful. Thanks also to my other committee members- Marcia Hobbs, Peter Leone, Pia MacDonald and Arlene Sena-Soberano-for sharing their expertise. My committee members' dedication to public health is truly impressive. I am very fortunate for the friendship of Rich MacLehose, who has generously and cheerfully helped me throughout the doctoral program.

I would like to thank the women who participated in this study and the group of professionals who conceptualized and carried it out. I am also appreciative for predoctoral funding through the U.S. Department of Health and Human Services Public Health Service Public Health training grant.

The love and support of my parents, Barbara and Raymond Thibeault, has been invaluable. I am also grateful for Nancy Hamilton and Molly Dean's encouragement. I must thank my children, Lazlo and Liska, for demanding balance in my life and for bringing me unparalleled joy each day. Finally, I owe the biggest thanks of all to my husband, Brad Torok. His unflagging support has enabled me to pursue this academic journey while enjoying our wonderful life together.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ABBREVIATIONS

AOR	adjusted odds ratio
BV	bacterial vaginosis
CI	confidence intervals
DP	Depo-Provera
EIA	enzyme immunoassay
HR	hazard ratio
H_2O_2	hydrogen peroxide
HIV	human immunodeficiency virus
IUD	intra-uterine device
LCR	ligase chain reaction
LMP	last menstrual period
LR	likelihood ratio
OC	oral contraceptive
OR	odds ratio
PID	pelvic inflammatory disease
STI	sexually transmitted infection
STD	sexually transmitted disease
Tv	*Trichomonas vaginalis*
UAB	University of Alabama
UNC-CH	University of North Carolina at Chapel Hill
US	United States

CHAPTER 1: INTRODUCTION

Trichomoniasis is thought to be the most common curable sexually transmitted infection (STI) in the world [2]. The prevalence of *Trichomonas vaginalis* (*Tv)* infection in women in the United States (US) ranges from 6%-54%, depending on the population being studied [3]. In US men, the range of prevalence is between 3% and 58%, although there have been fewer studies of men compared to women [3]. Health complications related to trichomoniasis for both men and women exist, and infection with *Tv* is a risk factor for human immunodeficiency virus (HIV) transmission [4]. Despite the public health importance of this infection, until recently trichomoniasis has received little clinical and research attention. For example, though it is known that the vaginal microenvironment can influence acquisition, presence, clinical presentation and detection of STIs, this has not been well-studied in trichomoniasis. For this dissertation, we examined the association between trichomoniasis in women and two factors that affect the vaginal microenvironment: vaginal lactobacilli and estrogen.

Specific Aims

Specific Aim1

Evaluate the association between *Lactobacillus* spp. vaginal Gram stain score and trichomoniasis

Hypothesis: Decreased *Lactobacillus* spp. vaginal Gram stain score is associated with increased frequency of trichomoniasis.

Overview: We compared *Lactobacillus* spp. vaginal Gram stain scores between women with and without trichomoniasis.

Rationale: Evaluation of the association between *Lactobacillus* spp. vaginal Gram stain score and trichomoniasis could improve our understanding of this infection and may have implications for management of trichomoniasis.

Specific Aim 2

Assess the association between exogenous estrogen and trichomoniasis among women

Hypothesis: Trichomoniasis diagnosis is less frequent among women using estrogen-containing contraceptives.

Overview: We compared OC use between women who tested negative for *Tv* and those who tested positive for the pathogen.

Rationale: Previous studies have reported conflicting results about OC use and risk of trichomoniasis [5-8] as well as the role of estrogen in *Tv* virulence [9, 10]. Assessing the impact of contraceptive use on STI status is important because it may influence a woman's contraceptive choice. Furthermore, contraceptive use may potentially influence transmission of HIV type 1 infection, since trichomoniasis is a risk factor for HIV.

Background and Significance

Once believed to be a benign infection, trichomoniasis—the world's most common non-viral STI—is now recognized as a major health problem. Fifteen years ago, a well-known medical text stated the effect of trichomoniasis on pregnancy was minimal, the cancer-causing potential for *Tv* was insignificant and that no clinical sequlae to the infection were known [11]. However, current research shows a relationship between trichomoniasis and many health problems in women including:

- postoperative infections
- tubal infertility
- pelvic inflammatory disease (PID)
- preterm birth
- premature rupture of the membranes
- low birth weight and
- cervical cancer [12-18]

Trichomoniasis has also been identified as a risk factor for HIV-1 transmission [4, 19, 20]. *Trichomonas vaginalis* may amplify HIV transmission by augmenting HIV shedding, expanding systemic access through lesions, and increasing the number of HIV target cells [4, 19, 21]. This suggests a substantial impact on HIV incidence among subgroups, such as black women, in whom trichomoniasis is common [19].

In 1999, the estimated annual incidence of trichomoniasis worldwide was approximately 173 million new cases [22, 23]; in the US, there were an estimated 7.4 million new cases in 2000 [24]. Prevalence estimates vary according to the population studied, from 3% in a student health center to 48% in a juvenile detention center [19]. In general, prevalence in US STD clinics is thought to be about 25%

[15]. First described almost 200 years ago, only now is trichomoniasis beginning to receive the attention it deserves as a major health hazard [11].

Vaginal lactobacilli and trichomoniasis

How the local vaginal milieu affects the occurrence of trichomoniasis is not known. Lactobacilli, the predominant microorganism in the healthy human vagina, are affected by many factors: sexual behavior, hormones, presence of blood, presence of foreign bodies (e.g. tampons) and medications/preparations (e.g. antibiotics or douching agents) [25, 26]. All lactobacilli produce lactic acid and some produce hydrogen peroxide (H_2O_2). Production of these compounds, via anaerobic metabolism of vaginal glycogen by lactobacilli, may protect against cervico-vaginal infection and may influence virulence of cervico-vaginal pathogens [27-29]. Thus, women with decreased or absent lactobacilli may be more likely to acquire or have cervico-vaginal infections.

Furthermore, hormones can affect the vaginal microenvironment, but this complex relationship is not well understood [30]. Currently, we know only that biological interaction between endogenous and exogenous sex hormones and trichomoniasis may occur, potentially impacting the presence of disease.

Epidemiologic evidence supports a relationship between vaginal lactobacilli and other cervico-vaginal conditions. In prospective studies, acquisition of bacterial vaginosis (BV) (hazard ratio [HR]=4.0, $p<0.001$), gonorrhea (HR=1.7, 95% CI: 1.1-2.6) and HIV-1 (HR=2.0, 95% CI:1.2-3.5) [26, 27] have been associated with lack of vaginal lactobacilli. Cross-sectional studies have also found associations between

decreased vaginal lactobacilli and presence of cervico-vaginal conditions including HIV-1, Group B streptococci, chlamydia and gonorrhea [31-33]. A number of studies have attempted to determine if H_2O_2 -producing lactobacilli inhibit growth of genital microorganisms.

Results from a cross-sectional study among a cohort of pregnant women showed those colonized by H_2O_2-positive lactobacilli were less likely to have BV and symptomatic candidiasis compared to women with H_2O_2-negative lactobacilli ($p<0.05$). Researchers from the same study also observed that women with H_2O_2-positive lactobacilli were less likely to have chlamydia compared to women with no vaginal lactobacilli ($p<0.05$) [31]. Similarly, in a longitudinal study conducted in women attending an STI clinic, acquisition of BV was associated with lack of vaginal H_2O_2-positive lactobacilli (HR=4.0, $p<0.001$) and presence of only H_2O_2-negative lactobacilli (HR=2.2, $p=0.02$) [27]. However, investigators from another longitudinal study failed to find a statistically significant association between acquisition of gonorrhea or HIV-1 among women with only H_2O_2-negative lactobacilli compared to women colonized with H_2O_2-positive lactobacilli. They did report an association between acquisition of HIV-1 among women without vaginal lactobacilli compared to women colonized with H_2O_2-positive lactobacilli (HR=2.8, 95% CI: 1.1-4.7) [26].

Whether vaginal lactobacilli level is associated with trichomoniasis is not known. *In vitro* and clinical data suggest an association between the two vaginal microorganisms. Research has shown that addition of lactobacilli to *Tv* culture slows growth of the protozoa [34]. *T. vaginalis* is also known to phagocytize

lactobacilli [35, 36]. Furthermore, lactobacilli have been used to treat trichomoniasis in women, although this remedy may not be efficacious [37, 38].

The epidemiologic data concerning trichomoniasis and vaginal lactobacilli also suggest an association but are not clear. Trichomoniasis was associated with an alkaline vaginal pH (resulting from decreased numbers of lactobacilli) in several early studies [39, 40]. This association was confirmed in univariable analyses of several later studies [41-44]. For example, Hillier et al (1991) reported pregnant women with lactobacilli-predominant vaginal flora were less likely to be infected with *Tv* compared to women with reduced lactobacilli or BV ($p< 0.001$) [32]. The most compelling results came from a cross-sectional study among pregnant women at a prenatal clinic in South Africa. In this study, women with decreased levels of vaginal lactobacilli were much more likely to have trichomoniasis compared to women with higher levels of vaginal lactobacilli (OR=7.8, 95% CI: 4.0-15.2) [41]. These studies suggest an association between vaginal lactobacilli and trichomoniasis.

Researchers investigating H_2O_2 -producing lactobacilli and STIs have also examined trichomoniasis. In a cross-sectional study, women with H_2O_2-negative vaginal lactobacilli were as likely to be colonized by *Tv* as women without vaginal lactobacilli. However, a statistically nonsignificant trend toward increased frequency of infection in women with H_2O_2-positive, H_2O_2-negative and no vaginal lactobacilli, respectively, was observed [31]. In a prospective cohort study, presence of H_2O_2-positive lactobacilli (compared to no vaginal lactobacilli and presence of H_2O_2-negative vaginal lactobacilli) did not appear to protect against acquisition of trichomoniasis [27]. Similarly, a prospective cohort study among sex workers in

Africa failed to find a statistically significant association between absence of lactobacilli compared to presence of H_2O_2-positive lactobacilli and acquisition of trichomoniasis (HR=1.4, 95% CI: 0.9-2.1) [26]. Therefore, an association between vaginal lactobacilli and trichomoniasis is questionable.

Discrepant study results may be due to use of different *Tv* diagnostic methods: wet mount microscopy [26, 39, 45], *Tv* culture [27, 40, 43], wet mount microscopy and *Tv* culture [31, 32, 42, 44, 46] and Pap smear [41]. Some of these studies suffered from small sample sizes as well. For example, one study in which vaginal lactobacilli did not appear to protect against acquisition of trichomoniasis included only 7 cases of the infection, another had only 27 cases [27, 31]. Another explanation for inconsistent results is that most studies failed to assess the influence of covariables on the relationship between vaginal lactobacilli and trichomoniasis. For example, age, race, date of last vaginal sex, douching frequency, douching preparation, OC use, menstruation and time in menstrual cycle may affect this association [5, 7, 25, 45-49]. Several studies considered some of these factors, but no study to date has considered all of them [25-27]. Finally, some of these studies included only pregnant women [32, 41]. A study in which non-pregnant women are included, potentially important covariables are considered and which uses the diagnostic reference standard will help elucidate the relationship between vaginal lactobacilli and trichomoniasis.

Estrogen and trichomoniasis

Another unresolved question about trichomoniasis and the vaginal microenvironment concerns the influence of sex hormones. Oral contraceptive use has been positively associated with chlamydia cervicitis and vaginal candidiasis in prospective cohort, case-control and cross-sectional studies [6, 8, 50, 51]. Two such studies adjusted for potential confounding variables such as sexual activity [6, 7]. These findings may be due to an increased number of endocervical epithelial cells available to *C. trachomatis* for attachment in OC users, since OC use is associated with cervical ectopy [30]. Furthermore, studies using animal models suggest that sex hormones enhance growth of *C. trachomatis* [6].

In contrast, use of OCs is thought to have an inverse relationship with BV and some PID-associated organisms [8, 30, 50, 51]. Depo-Provera (DP) may also be inversely associated with BV [6]. Oral contraceptive use may protect against PID-associated pathogens or may decrease susceptibility to some pathogens by thickening the cervical mucous [30, 52]. Modulation of vaginal infections and their clinical severity by endogenous hormones has also been implicated. Vulvovaginal candidiasis and *Mycoplasma genitalium* are present more often, and candidiasis symptoms are exacerbated, during certain times of the menstrual cycle [53, 54].

Conflicting results concerning the effect of estrogens on trichomoniasis have been reported. *In vitro* data have shown that *Tv* growth and cytoadherance are decreased in the presence of estrogen [55]. Epidemiologic data are consistent with these *in vitro* observations. An inverse relationship between trichomoniasis and OC use was reported by investigators using different study designs and both wet mount microscopy and *Tv* culture-confirmed diagnoses [5, 7, 46, 56-59]. In a prospective

cohort study that adjusted for covariables such as sexual behavior and demographics, OC users had 0.56 times the risk of acquiring trichomoniasis compared to women with an intra-uterine device (IUD) or surgical sterilization (95% CI: 0.39-0.81) [7]. Three other studies employed only univariable analyses, but also found that OC users were less likely to have trichomoniasis compared to non-OC users and users of other contraceptive methods [5, 46, 57]. Although it has not been well researched, one study found an inverse relationship between trichomoniasis and DP, a contraceptive containing progesterone [6]. Collectively, these data imply exogenous sex hormones may protect against trichomoniasis.

Conversely, *in vitro* data have shown that estrogen increases growth of *Tv* and that estrogenization is required to establish infection in animal species that are not natural hosts for the pathogen [60-62]. One study reported that a high estrogenic state was required to establish *Tv* infection in castrated human volunteers [60]. Finally, a clinical case report supports this hypothesis; a metronidazole-allergic postmenopausal woman was cured of trichomoniasis upon discontinuation of estrogen replacement therapy [63]. Positive associations between estrogen and *Tv* have not been reported in epidemiologic studies. However, some research has found no statistically significant difference in trichomoniasis between OC users and non-users [6, 8, 46, 50, 64-66]. These studies also used different designs and diagnostic methods, and only 1 performed multivariable analyses. This evidence questions the existence and/or direction of an association between exogenous estrogen and trichomoniasis.

Biological interaction of *Tv* and estrogen is physiologically plausible and some epidemiologic evidence implies that OC use may protect against acquisition of trichomoniasis. No study to date has fully explored the relationship between exogenous sex hormones and trichomoniasis using reference standard diagnostics while examining potentially important covariables.

Summary

Research presented in this dissertation provides insight into the interrelationship between trichomoniasis and the vaginal microenvironment. Use of the diagnostic reference standard, adequate sample size and consideration of covariables for effect measure modification and confounding are an improvement over many prior studies on these topics. The association of vaginal lactobacilli and trichomoniasis could have implications for *Tv* management, while the effect of OC on trichomoniasis may influence a woman's contraceptive choice and might have implications for the HIV epidemic.

CHAPTER 2: RESEARCH DESIGNS AND METHODS

Specific Aim 1

Evaluate the association between *Lactobacillus* spp. vaginal Gram stain score and trichomoniasis.

Study design: Overview

Data were obtained from the prospective case-control study "*Trichomonas vaginalis* Infection in the Sexual Partners of Infected Women", which was conducted from 2001 to 2003 among female patients and their male sexual partners presenting to sexually transmitted disease (STD) clinics at three public health departments located in North Carolina and Alabama. Five hundred thirty-nine women with trichomoniasis and 177 uninfected women participated in the study. Trichomoniasis was diagnosed in women who had either a positive wet mount microscopy or *Tv* culture result. Women were considered to be *Tv*-negative if both wet mount microscopy and *Tv* culture were negative. All study participants underwent a routine medical history and evaluation by research staff and completed a provider-administered questionnaire. This study was approved by the University of North Carolina at Chapel Hill (UNC-CH) and University of Alabama (UAB) Institutional Review Boards.

Study setting

Durham and Wake counties, NC and Jefferson County, AL STD clinics serve socio-demographically similar patients. The racial/ethnic composition is approximately 75% black, 15% white and 10% Hispanic. The median patient age for women is 25 years and the estimated prevalence of *Tv* infection among women ranges from 16-22%.

Study population

Demographics: Most women in the study attended the Jefferson Country Health Department (55%), the others were roughly divided between the two North Carolina health departments. The majority of women in the study were black (91%) and most were less than 35 years of age (65%). A sizeable portion (35%) was not employed, and most female participants had never been married (74%). Approximately half the participants had a household income in the previous year less than $12,000, and less than half (34%) obtained education beyond high school.

During the physical exam, clinicians found at least one clinical sign suggestive of an STI in many women. The most common sign was abnormal vaginal discharge (79%), followed by abnormal cervical discharge (17%) and abnormal vaginal erythema (14%). Clinical specimens for STI testing were obtained from consenting women. The most common STIs were chlamydia (9%) and gonorrhea (8%).

Recruitment: All female patients were offered prescreening for the study based on criteria listed below:

- At least 18 years of age
- English-speaking
- No prior enrollment in the study
- Verbal consent to *Tv* culture
- Verbal consent to vaginal Gram stain

Women with positive wet mount microscopy results were recruited to participate in the study. Following recruitment of every third woman positive for trichomoniasis, the next prescreened woman with negative wet mount microscopy results was recruited to participate. Study inclusion was dependent upon the following criteria:

- At least 18 years of age
- English-speaking
- No prior enrollment in the study
- History of vaginal sex with men in the past 60 days
- No treatment with topical or oral metronidazole in the 4 weeks prior to *Tv* culture collection
- A positive wet mount microscopy or culture result for *Tv*, or
- Selection as a *Tv* negative control
- Written consent for the study

Study exclusion criteria were as follows:

- History of treatment with topical or oral metronidazole in the 4 weeks prior to *Tv* culture collection

- A history of domestic violence with current sexual partners
- Pregnancy
- Prior prescreening/enrollment in the study

Data collection

Medical History: Research personnel asked all participants about their medical history. The medical history covered the following topics: reason for visit, STI history, number of sexual partners in past 60 days, number of sexual encounters in past two weeks and condom use.

Physical Exam: Research staff performed a physical exam on all participants. During the physical exam, inguinal lymph nodes, vulva and perineum were inspected for lesions, the vagina was evaluated for discharge and erythema, the cervix was evaluated for discharge and friability and a bimanual pelvic exam was performed to assess cervical motion or adnexal tenderness. A vaginal swab for wet mount microscopy and Gram stain was collected and vaginal pH was measured using a pH strip. Additional STI tests were offered to all participants as per standard clinic protocols.

Questionnaire: In addition to the routine medical history and physical exam, a questionnaire was administered by trained interviewers to all participants in a confidential location. Topics discussed in the questionnaire included reason for the visit, potential barriers to seeking medical care, STI history, current symptoms, demographic information such as age, race/ethnicity, level of education, income, sexual history, sexual behaviors, first day of last menstrual period (LMP) and

douching behaviors. Interviewers were unaware of the infection status of the participants.

During the interview, each participant was asked about contraceptive use during vaginal sex in the past two months with their most frequent and most recent partner(s). Women were asked specifically about the following contraceptive methods: IUD, OC, DP injections and Norplant. Each participant was also asked "When was the first day of your last menstrual period?" To obtain douching information, participants were asked "Have you douched in the last two months?", "How many times have you douched in the last week?" and "How many times do you douche in an average or usual week?" Participants were also asked what kind of preparation they usually used when they douche.

Laboratory Evaluation: For wet mount microscopy, a vaginal swab was obtained according to routine standard of care during speculum examination. Vaginal swabs were placed into a sterile tube containing 0.5 mL of normal saline upon collection. The tube was agitated in the laboratory and a drop of the solution was then placed on a glass slide, covered with a cover slip and examined under the microscope at 200X magnification.

All study participants were offered chlamydia, gonorrhea, syphilis and HIV testing per standard clinic protocol. Chlamydia testing was performed using enzyme immune assay, DNA hybridization or ligase chain reaction (LCR). One clinic performed enzyme immunoassay (EIA) for *C. trachomatis* on all female patients; the others performed LCR for detection of *Chlamydia trachomatis* for women under the age of 25 years. Assays were performed according to the manufacturers'

instructions. *N. gonorrheae* was detected by culture or DNA hybridization. Syphilis testing was conducted using standard serologies (rapid plasma regain and microhemagglutination assay for *Treponema pallidum*). HIV tests were performed by the state laboratories.

For *Tv*, a vaginal swab was placed in a 15 mL conical centrifuge tube with a screw-cap for delayed inoculation into the InPouch culture (Biomed Diagnostics, San Jose, CA). InPouch cultures were read each day by research personnel at each health department until transport to the research labs at UNC-CH or UAB. Cultures were read for 5 days. Using the 20X objective, at least 5 microscopic fields were examined per pouch at each reading.

For Gram stain of vaginal flora, a vaginal smear was obtained by rolling a swab across the vaginal wall and then onto a glass slide. Vaginal smears were sent to the UAB research lab on a monthly basis. The smears were fixed and stained, and each slide was evaluated for large Gram-positive rods (indicative of lactobacilli) under oil immersion (1000X magnification) and interpreted using criteria described by Nugent [1].

Measurements

Exposure*: Lactobacillus* spp. vaginal Gram stain score: *Lactobacillus* spp. vaginal Gram stain score was determined, using criteria previously described in the literature [1]. This variable was dichotomized into two groups: ≥30 large Gram-positive rods (*Lactobacillus* spp. morphotypes) per oil immersion field vs. ≤30 large Gram-positive rods (*Lactobacillus* spp. morphotypes) per oil immersion field.

Bivariable analyses were performed in parallel with *Lactobacillus* spp. vaginal Gram stain score coded as an indicator and dichotomous variable. Coding did not appreciably change the results, so dichotomous coding was used in multivariable analyses to optimize precision and ease of interpretation.

Outcome: Trichomoniasis diagnosis using the reference standard: The presence of at least one motile trichomonad at any culture reading defined a positive culture result. The absence of motile trichomonads at all culture readings defined a negative culture result. Specimens with motile trichomonads observed by wet mount microscopy were classified as a positive wet mount microscopy result. Those without motile trichomonads were classified as a negative wet mount microscopy result. The primary outcome, diagnosis of trichomoniasis, is a dichotomous variable. Women with positive wet mount microscopy or *Tv* culture results were classified as having trichomoniasis. Women with negative wet mount microscopy results and a negative *Tv* culture result were classified as not having trichomoniasis.

Covariables: Age was calculated from the study date and date of birth. It was initially coded as a continuous variable and then categorized into clinically reasonable age groups. Race/ethnicity was originally coded categorically using indicator variables for white, black and other race/ethnicity. It was then dichotomized into black and other race/ethnicity. The highest level of education for each participant was ascertained. This information was dichotomized for women with ≤high school education and women with >high school education. To measure household income, each participant was asked how much money people in their household brought in (before taxes) in the previous year. This covariable was then

dichotomized into <$12,000 and >$12,000. Level of endogenous estrogen was estimated using the qualitative marker of time during menstrual cycle. The interviewer recorded the day, month and year of the LMP reported by the participant. Since there is no accepted definition of high versus low level of estrogen throughout the menstrual cycle, and responses to this question from an expert panel of gynecologists differed, this variable was assessed in several ways. Ultimately, the coding schemes did not substantially change the results, so we dichotomized this variable into menstrual cycle days 6-14 ("higher estrogen") and menstrual cycle days 1-5 and 15-28 ("lower estrogen" days).

Gonorrhea, chlamydia, syphilis and HIV were diagnosed according to the methods described previously. Yeast was diagnosed by wet mount microscopy and BV was diagnosed by the Nugent Gram stain method [1]. These covariables were coded dichotomously. All participants were asked if they had ever been diagnosed with gonorrhea, chlamydia, syphilis, trichomoniasis, BV, cervicitis, PID, genital warts. genital herpes and HIV. Each STI covariable was coded dichotomously. Whether or not the patient had douched in the past 2 months was included as a dichotomous covariable. Number of times participants douched in the past week was also considered. The following categories of usual douching preparation were also examined as a nominal variable: water only, water plus baking soda, water plus vinegar, store bought preparation and other preparation. The interviewer indicated a positive or a negative response to use of each type of contraception. Sexual activity was assessed using dichotomized variables of the number of different sex partners a

woman had in the past year and the number of times she has sex in an average week.

Data management and data analysis

Data management*:* The data were collected on three ply paper from the NCR Corporation. Two plies were sent to the data management group and one ply remained at each site. All required data were entered using a validated clinical database management system with double data entry.

Data analysis: We examined the frequency distribution of trichomoniasis diagnosis, *Lactobacillus* spp. score and categorical covariables. Measures of central tendency and variability were also assessed for continuous variables. Additionally, we reviewed the data for missing and unusual values as well as extreme distributions.

Bivariable data analysis is useful for identifying potential effect modifiers and confounding variables. Since a dose-response relationship between decreasing categories of *Lactobacillus* spp. score and diagnosis of trichomoniasis is biologically reasonable, we performed a chi-square test for trend in order to assess a linear relationship between the two variables. Also, the assumption of linearity in the logit was assessed in this manner for all ordinal and continuous variables in the analysis. Next, we evaluated potential effect modification using stratum-specific odds ratios (OR)s to assess substantive differences and the Breslow-Day Test for Homogeneity to appraise statistically significant differences between the estimates across levels of covariables. The OR and 95% CI for the association between *Lactobacillus* spp.

vaginal Gram stain score and being diagnosed with trichomoniasis was estimated at each level of every covariable. Differences in estimates were informally compared, and whether or not the 95% CI included the estimate from an adjacent stratum was evaluated. Estimates were then tested for statistically significant differences using the Breslow-Day Test set at α=0.20. Results indicating substantive differences and which resulted in p-values < 0.10 were considered as effect modifiers in the multivariable analyses.

Variables that met the criteria to potentially confound the exposure-outcome relationship were assessed using a stratified tabular approach [67]. Crude and adjusted ORs (OR_{crude}- $OR_{adjusted}$ / $OR_{adjusted}$) were compared, and those variables for which the difference was > 0.10 were considered to potentially confound the main association.

Age, race/ethnicity, high/low estrogen day of menstrual cycle, other STI diagnosis, douching in previous week, number of times douches in usual week, douching preparation usually used and OC use were all considered for potential effect measure modification and/or confounding for specific aim 1.

Multivariable analyses

Multiple logistic regression was performed. A backward elimination model building strategy was used, which allows for assessment of the joint effects by covariables. The starting (full) model included the exposure, outcome and all covariables as well as exposure-covariable interaction terms created based on results from the bivariable analyses. Interaction terms were assessed first by

creating a reduced model (without the interaction term) and comparing likelihood ratio (LR) tests from the two models. The ORs from the two models were also informally compared for substantive differences. If the LR p-value was ≤ 0.10 and the interaction term was deemed substantively different, the interaction term was retained in the model. If the LR p-value was > 0.10 and the term was deemed to not be substantively different, the interaction term was dropped from the model. The order in which interaction terms were evaluated was based on their p-values (the term with the largest p-value was assessed first).

Next, potential confounding was assessed by comparing the crude and adjusted OR for each potential confounding variable. We considered covariables resulting in $|\ln(OR_{crude}/OR_{adjusted})| > 0.10$ as potential confounders and these were retained in the model. Otherwise the covariables were dropped from the model. Again, the order in which covariables were assessed for confounding based on their p-value was the same as described above for effect modification.

The final model contained the exposure and outcome variables along with any covariables identified as effect modifiers (and their interaction terms) and those identified as potentially confounding the exposure-outcome association.

Summary: Specific aim 1

This aim utilized multiple logistic regression analysis to examine the association between level of vaginal lactobacilli and prevalent *Tv* infection in women.

Specific Aim 2

Assess the association between oral contraceptive use and trichomoniasis among women.

Study design

Please refer to study design, specific aim 1.

Study setting

Please refer to study setting, specific aim 1.

Data collection

Please refer to data collection, specific aim 1.

Measurements

Please refer to measurements, specific aim 1.

Data management and analysis

Please refer to data management and analysis, specific aim 1.

Summary: Specific aim 2

This aim compared OC use between women with and without trichomoniasis.

CHAPTER 3:

THE ASSOCIATION BETWEEN *TRICHOMONAS VAGINALIS* INFECTION AND LEVEL OF VAGINAL LACTOBACILLI AMONG NON-PREGNANT WOMEN

Abstract

Background

Trichomoniasis is an important public health issue. Many factors influencing acquisition, presentation and pathogenesis of Tv are not understood. The vaginal microenvironment and lactobacilli affect many STI, but their impact on trichomoniasis is not known.

Setting

Three STD clinics located in public health departments in the Southeast US.

Methods

Five hundred twenty one consenting female clinic patients who met eligibility criteria and were positive for trichomoniasis were recruited into the study. The control group of 176 women was selected from symptomatic and asymptomatic women who were not diagnosed with trichomoniasis who presented to the clinics for evaluation during the study period. All study participants underwent physical examination and routine STI testing. An in-person questionnaire was also administered to gather demographic and behavioral information.

Results

After adjusting for age, race, education, concurrent BV, concurrent gonorrhea and/or syphilis, number of sex partners in the past 2 months, number of times the woman has sex in an average week, and OC use, lower levels of vaginal lactobacilli were positively associated with Tv among women with greater than a high school education (OR=4.6; 95% CI, 2.2-9.7). Trichomonas vaginalis was not independently associated with lower levels of vaginal lactobacilli among women with less than or equal to a high school education after adjusting for potentially confounding variables (OR=1.5; 95% CI, 0.7-3.5).

Conclusion

Vaginal lactobacilli may be associated with trichomoniasis in subgroups of women. Whether lower levels of vaginal lactobacilli predispose women to trichomoniasis or *Tv* infection leads to lower levels of lactobacilli is not known.

Introduction

Trichomoniasis is the most common curable STI world-wide [2]. The prevalence of *Tv* infection in women in the US ranges from 6%-54%, depending on the population [3]. Trichomoniasis can lead to health problems such as PID and preterm delivery in women [12, 16]. Furthermore, *Tv* is a risk factor for HIV [4]. Until recently, trichomoniasis has received little clinical and research attention, despite its public health importance. While the vaginal microenvironment is known to influence other STIs, it has not been well-studied in trichomoniasis [31-33].

Lactobacillus species are the most prevalent organism in normal human vaginal flora and may protect the vagina against infection [27]. Lactobacilli produce antimicrobial compounds as well as lactic acid, creating an environment that is inhospitable to some pathogenic bacteria [31]. The association between *Tv* and lactobacilli is not understood. The addition of lactobacilli to *in vitro Tv* culture slows growth of the protozoa and *Tv* phagocytizes lactobacilli [34-36]. However, epidemiological evidence is inconsistent. In several early studies, trichomoniasis was associated with an alkaline vaginal pH, indicating low levels of vaginal lactobacilli [37, 39, 40, 68]. Later studies also suggest an association between vaginal lactobacilli and trichomoniasis [41-44]. However, other studies found no association between vaginal lactobacilli and trichomoniasis [25, 26].

We conducted a case-control study to assess the association between vaginal lactobacilli and *Tv* among non-pregnant women. Clarifying this relationship could improve our understanding of *Tv* and may have implications for treatment of trichomoniasis.

Methods

Study design and sample

We conducted a prospective, case-control study in 3 health department STD clinics located in Durham and Raleigh, North Carolina and Birmingham, Alabama from November 2001 to July 2003. Female patients who were ≥18 years old, spoke English and had not previously been prescreened or enrolled in the study were eligible for prescreening. Prescreened patients with positive *Tv* wet mount

microscopy results were recruited immediately. Prescreened patients with negative *Tv* wet mount microscopy results but positive *Tv* culture results were requested to return for treatment and/or counseling and were recruited for the study during that clinic visit. The control group was selected from symptomatic and asymptomatic women presenting to the STD clinics for evaluation during the study period and were defined as women found to be uninfected with *Tv* by wet mount. Patients who were subsequently diagnosed with trichomoniasis by culture were reclassified as a case. We recruited 1 woman with a negative *Tv* wet mount microscopy result for every third subject with a positive wet mount microscopy enrolled in the study. Recruited women who had a history of vaginal sex within past 60 days and had not used metronidazole in the 4 weeks before *Tv* culture collection were eligible for study enrollment. All enrolled participants provided written informed consent. The study was approved by the UNC-CH and the UAB Biomedical Institutional Review Boards.

Clinical, demographic and behavioral data

Two vaginal swabs, 1 for Gram stain and 1 for *Tv* culture, were obtained from prescreened women. Vaginal wet mount and routine testing for *N. gonorrheae* and syphilis were performed. Routine testing for *C. trachomatis* was also performed, but 1 clinic limited it to women aged ≤25 years. All female subjects underwent a physical examination involving their inguinal lymph nodes, vulva, perineum, vagina and cervix, and a bimanual pelvic examination assessing for cervical motion or adnexal tenderness. Clinical information was obtained from their medical records. Furthermore, research personnel administered detailed questionnaires about

demographics, clinical symptoms, STI history, douching behavior, sexual behavior and contraceptive use.

Laboratory Analyses

Wet mount microscopy was performed according to routine clinic procedures by trained laboratory personnel at each site. *T. vaginalis* culture was performed using the InPouch *TV* culture system (Biomed, White City, OR). Cultures were read daily up to 5 days following inoculation. Vaginal smears were sent to UAB for Gram Stain evaluation and were interpreted using Nugent's criteria [1]. Gonorrhea testing was performed using culture or DNA hybridization. Syphilis testing was conducted using standard serologies (rapid plasma reagin). Confirmatory syphilis testing was performed with microhemagglutination assay for *Treponema pallidum.*

Statistical Analyses

All data were double-entered by data management personnel into a SAS version 8 database (SAS Corporation, Cary, NC). We conducted descriptive analyses of the study population by examining the frequency distribution of *Tv*, *Lactobacillus* spp. score on the vaginal Gram stain and categorical covariables. Measures of central tendency and variability were assessed for continuous covariables.

We defined trichomoniasis, the primary study outcome, based on positive wet mount microscopy or *Tv* culture results. Women with negative wet mount

microscopy results and negative *Tv* culture results were defined as not being infected with *Tv*.

The level of vaginal lactobacilli was measured by the *Lactobacillus* spp. vaginal Gram stain score using criteria described in the literature [1]. We classified women who had ≥30 lactobacillus morphotypes per oil immersion field (*Lactobacillus* spp. vaginal Gram stain score of 4+) as having "high" levels of vaginal lactobacilli. Women who had 0 to 30 lactobacillus morphotypes per oil immersion field (*Lactobacillus* spp. vaginal Gram stain score of 0 to 3+) were classified as having "low" levels of vaginal lactobacilli [1].

We used bivariable analyses to evaluate potential effect measure modification and confounding of the association between vaginal lactobacilli level and *Tv*. Effect measure modification was assessed using the Breslow-Day Test of Homogeneity. Variables that indicated substantive differences and resulted in p-values <0.20 were assessed in the multivariable analyses for effect measure modification. Variables that were associated with both *Lactobacillus* spp. vaginal Gram stain score and *Tv* were considered to potentially confound this association. We evaluated these variables using a stratified tabular approach [67].

To describe the association between level of vaginal lactobacilli and infection with *Tv* in multivariable analyses, an adjusted OR and 95% CI were estimated using unconditional multiple logistic regression.

We used a backward elimination model building strategy. Interaction terms were assessed by comparing the LR for the models with and without the interaction term. Variables resulting in LR p-value ≤0.10 were retained in the model.

Confounding of the association between *Lactobacillus* spp. vaginal Gram stain score and *Tv* was assessed by comparing the fully adjusted OR to the adjusted OR after removal of potentially confounding variables until the cumulative change in the OR of the exposure was >10%. We evaluated variables in order of the magnitude of the Wald chi-square test p-value and did not include variables with >20% missing data in the multivariable analyses.

Results

Study population

Seven hundred ninety women were diagnosed with *Tv*. Of these, 539 (68.4%) infected women participated in the study and 521 (66.0%) were included in the present analyses (18 were excluded due to missing data). Of the 203 women negative for *Tv* recruited for the study, 177 (87.2%) agreed to participate and one was excluded due to missing data.

The median age of participants was 27 years old (standard deviation, 9 years) and most were non-Hispanic Black (91%) (Table 3.1). Half reported a household income of ≥$12,000 in the previous year and 35% had graduated from high school or had passed the General Educational Developmental test. Almost half (48%) of the participants had douched in the past 2 months. Of those who had, most used a commercial preparation (75%).

Association between vaginal lactobacilli level and T. vaginalis

Low levels of vaginal lactobacilli were positively associated with *Tv* in bivariable analyses (OR=2.2; 95% CI: 1.4-3.2) and remained so after adjusting for age, race, education, concurrent BV, concurrent gonorrhea and/or syphilis (a marker of sexual behavior), number of sex partners in the past 2 months, number of times the woman has sex in an average week, and OC use (Adjusted odds ratio [AOR]=2.8; 95% CI: 1.5-5.0).

Multivariable analyses suggested that level of education modifies the association between vaginal lactobacilli level and *Tv* (p=0.02, LR) (Table 3.2). Among women with greater than a high school education, low levels of vaginal lactobacilli were positively associated with *Tv* (OR=3.3; 95% CI: 1.8-5.9). This association was not observed in women with at most a high school education (OR=1.1; 95% CI: 0.6-2.0). After adjusting for the same cofactors as those in the bivariable analyses (except for educational level), low levels of vaginal lactobacilli remained positively associated with *Tv* among women with greater than a high school education (OR=4.6; 95% CI, 2.2-9.7). *Trichomonas vaginalis* was not independently associated with low levels of lactobacilli among women with less than or equal to a high school education after adjusting for potentially confounding variables (OR=1.5; 95% CI, 0.7-3.5). See Table 3.2 for these associations. Adjustment of the final model for chlamydia coinfection and for prior *Tv* infection did not substantially alter our results.

Discussion

Trichomoniasis is the world's most common curable STI and an apparent contributor to HIV acquisition [2, 4, 19]. Yet trichomoniasis remains poorly understood [2, 19, 69]. The relationship between vaginal lactobacilli and *Tv* remains unclear, though it could impact important aspects of infection. For example, abnormal vaginal flora following cervico-vaginal conditions and antimicrobial treatment of these conditions may promote infection relapse. Exogenously applied live lactobacilli and oestriol have been shown to help restore normal vaginal microflora [70]. A clearer understanding of the role of lactobacilli in the natural history of *Tv* may help refine this therapy and could possibly improve *Tv* treatment.

Anaerobic metabolism of vaginal glycogen by lactobacilli produces lactic acid and H_2O_2, and may influence virulence of pathogens [27-29]. For example, lack of vaginal lactobacilli has been positively associated with acquisition and presence of cervico-vaginal conditions, including BV, chlamydia, gonorrhea and HIV-1 [26, 27, 31-33]. Thus, women with decreased or absent vaginal lactobacilli may be more likely to acquire or have cervico-vaginal conditions.

The relationship between vaginal lactobacilli and *Tv* is not understood. *In vitro* data suggest an association between the vaginal microorganisms [34-36] while results from epidemiologic studies are inconsistent [25, 27, 32, 39-44, 68]. Discrepant study results may be due to use of different diagnostic methods to define *Tv* infection including wet mount microscopy [26, 39, 45], *Tv* culture [27, 40, 43], wet mount microscopy and *Tv* culture [31, 32, 42, 44, 46] and Pap smear [41]. Furthermore, most studies failed to comprehensively assess the influence of

covariables on the association between lactobacilli and *Tv* [5, 7, 25, 45-49]. Finally, some studies included only pregnant women, whose vaginal flora differs from non-pregnant women [32, 41, 71] while others were conducted with small sample sizes [27, 31].

In this study, lower levels of vaginal lactobacilli were positively associated with prevalent *Tv*. Association of vaginal lactobacilli level and *Tv* varied between women with different levels of education. Among women with a lower educational level (≤ high school), *Tv* was not independently associated with low levels of vaginal lactobacilli. Among women with greater than a high school education, *Tv* was associated with low levels of vaginal lactobacilli after adjusting for covariables that could potentially confound this relationship.

The reason for the observed difference in effect between educational levels is unclear. Education may be a proxy for another factor that we did not measure or did not measure well. Perhaps educational level is a marker for general health status. If this were the case women with lower educational levels (and relatively poorer health) may already have health problems, such as abnormal vaginal flora or general immune suppression, so that a reduction in vaginal lactobacilli would not be a strong enough biological insult to influence *Tv* status. However, in the higher educational group (with better general health) having lower levels of vaginal lactobacilli might be a strong enough biological insult to have an effect on *Tv* status. Given the relative imprecision of these education-specific estimates, this observation may also have been due to chance.

Douching has been associated with changes in vaginal flora and cervico-vaginal conditions, including *Tv*, making it an important variable to consider in studies of vaginal flora and trichomoniasis [25, 46, 72-75]. Despite this, there are limited data on this topic [25, 27]. We considered general douching frequency and preparation in our analyses, but neither variable appreciably affected the association between vaginal lactobacilli and *Tv*. Therefore, douching does not appear to explain the observed association in this study.

We were unable to explore the temporality of vaginal lactobacilli level and *Tv*. Although we observed an association between levels of vaginal lactobacilli and *Tv* among certain groups of women, prospective studies are needed to clarify whether low levels of vaginal lactobacilli are a risk factor for *Tv* acquisition or whether *Tv* causes levels of vaginal lactobacilli to decrease, perhaps via phagocytosis [35, 36].

Furthermore, vaginal flora are dynamic. Daily variability of vaginal flora has been associated with variables such as use of vaginal medication and spermicides [47]. Because of this, the level of lactobacilli measured in this study may not reflect the level of lactobacilli present when the participant was infected with *Tv*.

Several researchers have reported no association between H_2O_2-producing lactobacilli and *Tv*, though these studies relied on univariable analyses or were limited by sample size [26, 27, 31]. We were unable to examine this question because we did not identify strains of vaginal lactobacilli.

Finally, since *Tv* is not reportable nationally, its scope and demographics in the US are not definitively known, though it is thought to affect women with a demographic profile similar to those diagnosed with bacterial STIs. Women in this

study were recruited from 3 public STI clinics in the US Southeast and, therefore, may not accurately represent all US women infected with trichomoniasis. Although we cannot determine if these results are nationally representative, they may be representative of the most relevant regional population, since the Southeast US has a disproportionately high prevalence of STIs [76].

Our results suggest that lower levels of vaginal lactobacilli are associated with presence of *Tv* in subgroups of women, and that this relationship is not explained by other measured demographic, clinical or behavioral factors. Understanding this relationship may improve our knowledge of the pathogenesis, acquisition and management of trichomoniasis.

Table 3.1 Characteristics of women in the study

Characteristic	N (%)
Age, year	
18-24	273 (39.17)
25-29	132 (18.94)
30-34	95 (13.63)
35-39	86 (12.34)
40-44	74 (10.47)
≥45	38 (5.45)
Race/ethnicity	
Black, non-Hispanic	633 (90.82)
Other	64 (9.18)
Last years' household income	(n=648)
<$12,000	324 (50.00)
≥$12,000	324 (50.00)
Education	
≤ high school graduate or equivalent	454 (65.14)
> high school graduate or equivalent	243 (34.86)
Marital status	
Single, never married	514 (73.74)
Married	64 (9.18)
Separated	40 (5.74)
Divorced	73 (10.47)
Widow	6 (0.86)
Level of vaginal lactobacilli	
0-30/field	563 (80.77)
≥30/field	134 (19.23)
Number of times patient has sex/average week	(n=674)
≥1	614 (91.10)
0	60 (8.90)
Number of sex partners in past 2 months	(n=696)
≥2	219 (31.47)
≤1	477 (68.53)
Bacterial vaginosis status	
positive	362 (51.94)
negative	335 (48.06)
Coinfection with gonorrhea and/or syphilis	(n=647)
yes	65 (10.05)
no	582 (89.95)
Patient diagnosed with STI in past[1]	(n=694)
no/don't know	147 (21.18)
yes	547 (78.82)
Oral contraceptive use[2]	(n=693)
yes	79 (11.40)
no	614 (88.60)
Condom use[2]	(n=686)
yes	306 (44.61)
no	380 (55.39)
Douched in past 2 months	(n=694)
yes	336 (48.41)

no	358 (51.59)
Number of times patient douches/average week	(n=627)
≥1 time	86 (13.72)
0 times	541 (86.28)
Usual douching preparation[3]	(n=326)
commercial preparation	246 (75.46)
water+vinegar	67 (20.55)
other	13 (3.99)

[1]other than trichomoniasis
[2]with most recent and/or frequent sexual partner
[3]among women who douched in past 2 months

Table 3.2 Association between level of vaginal lactobacilli and *T. vaginalis* infection

Education	Vaginal lactobacilli	N	*T. vaginalis* + (%)	Odds Ratio (95% CI) Unadjusted	Adjusted[1]
Any educational level					
	High[2]	134	61.94	1.00	1.00
	Low[3]	563	77.80	2.15 (1.44-3.22)	2.75 (1.53- 4.96)
≤ high school graduate or equivalent					
	High	67	79.10	1.00	1.00
	Low	387	80.10	1.06 (0.56-2.02)	1.52 (0.67-3.47)
> high school graduate or equivalent					
	High	67	44.78	1.00	1.00
	Low	176	72.73	3.29 (1.83-5.90)	4.57 (2.15-9.72)

[1]Adjusted for race/ethnicity, age, diagnosis of bacterial vaginosis, coinfection with gonorrhea and/or syphilis, condom use with most frequent and/or recent sexual partner, oral contraceptive use, number of times has sex in average week, number of sex partners in past 2 months

[2]≥30/field

[3]<30/field

CHAPTER 4: ORAL CONTRACEPTIVE USE AND *TRICHOMONAS VAGINALIS* INFECTION IN WOMEN

Abstract

Background

Trichomonas vaginalis is the most common non-viral STI worldwide. Over 80 million women use OC, and OC are known to influence other STI. Some studies suggest OC use may be protective against *Tv*, while other studies found no protective effect.

Setting

Three STD clinics located in public health departments in the Southeast US.

Methods

Five hundred twenty one consenting female clinic patients who met eligibility criteria and were positive for trichomoniasis were recruited into the study. The control group of 176 women was selected from symptomatic and asymptomatic women who were not diagnosed with trichomoniasis who presented to the clinics for evaluation during the study period. All study participants underwent physical

examination and routine STI testing. An in-person questionnaire was also administered to gather demographic and behavioral information.

Results

The OR for OC use and trichomoniasis was 0.6, 95% CI 0.3-1.0, after adjusting for race/ethnicity, highest educational level attained, condom use, concurrent BV, level of vaginal lactobacilli, number of sex partners in the past year and number of times the woman has sex in an average week.

Conclusion

Oral contraceptive use appears to be moderately protective against *Tv*.

Introduction

Trichomonas vaginalis is the most common non-viral STI worldwide. Furthermore, *Tv* has been associated with health problems ranging from adverse pregnancy outcomes and cervical cancer to an increase in HIV transmission [2-4, 19, 59].

The effect of OC use on *Tv* is of particular interest because over 80 million women worldwide use OC. Oral contraceptive use affects the acquisition of other reproductive tract conditions, such as chlamydia, gonorrhea, candidiasis and BV [6, 8, 30, 50, 51, 58, 64, 77]. Results from previous studies on OC use and *Tv* are

conflicting [5-8, 46, 56, 57, 65, 66, 78]. This could be due, in part, to differences in diagnostic and analytic methods and study populations. The objective of this analysis was to clarify the relationship between OC use and prevalent *Tv* in non-pregnant women.

Methods

Study design and sample

This prospective, case-control study was conducted from November 2001 to July 2003. Participants were recruited from three STD clinics located in Durham and Raleigh, North Carolina and Birmingham, Alabama health departments. Eligible female patients for prescreening were English-speaking, ≥18 years of age, and had no history of previous prescreening or study enrollment. Women who were prescreened and had a positive *Tv* wet mount microscopy test were recruited into the study at that time. Women were eligible for study enrollment if they had not used metronidazole for 4 weeks prior to, and had a history of vaginal sex within 60 days of *Tv* culture collection. Women who were prescreened with negative *Tv* wet mount microscopy but positive *Tv* culture results were contacted to return to the clinic for treatment and/or counseling. These women were recruited into the study at their return visit. The control group was selected from symptomatic and asymptomatic women presenting to the STD clinics for evaluation during the study period, and were defined as women found to be uninfected with *Tv* by wet mount. Patients who were subsequently diagnosed with trichomoniasis by culture were reclassified as a case. We recruited 1 woman with a negative *Tv* wet mount microscopy result for

every 3 subjects with positive wet mount microscopy results enrolled in the study. All enrolled participants provided written informed consent. The study was approved by the UNC-CH and the UAB Biomedical Institutional Review Boards.

Clinical, demographic and behavioral data

Vaginal swabs for *Tv* culture and Gram stain were collected from prescreened women. A routine pelvic exam and vaginal wet mount were performed on all women. Routine testing for *N. gonorrheae* and syphilis were performed. Clinical, demographic and behavioral data were obtained from medical records and an in-person questionnaire administered by research personnel.

Laboratory Analyses

T. vaginalis culture was performed using the InPouch *TV* culture system (Biomed, White City, OR) and was read daily up to 5 days after inoculation. Laboratory personnel performed wet mount microscopy at each study site. Testing for gonorrhea was conducted by *N. gonorrheae* culture or DNA hybridization. Syphilis testing was performed using standard serologies (rapid plasma reagin and microhemagglutination assays for *T. pallidum*). Vaginal smear Gram stain was performed and evaluated at UAB, using Nugent's criteria [1].

Measures

We defined *Tv* infection as women with positive wet mount microscopy or *Tv* culture results. Women with negative wet mount microscopy results and negative *Tv* culture results were defined as not being infected with *Tv*.

We considered women who used OC with their most recent and/or most frequent sexual partner to be on OC. Women who did not use OC with their most recent and/or frequent sexual partner were classified as not being on OC.

Statistical Analyses

SAS version 8 (SAS Corporation, Cary, NC) was used for data analyses. All data were double entered. Univariable analyses of the study population were conducted using frequency distributions of categorical variables. Measures of central tendency and variability were assessed for continuous variables.

Variables assessed for potentially modifying or confounding the association between OC use and *Tv* included demographics (race/ethnicity, last year's household income, highest educational level attained), sexual behavior (past diagnosis with an STI other than *Tv*, concurrent diagnosis of gonorrhea and/or syphilis, condom use in the past 2 months, number of different sex partners in the past year and number of times the woman has sex in an average week), douching behavior (number of times in the past week, number of times in an average week) and factors related to the vaginal microenvironment (concurrent diagnosis with BV, level of vaginal lactobacilli).

We initially tested each covariable for EMM and confounding by creating a model with the exposure variable and one additional covariable, and comparing it to a model containing only the exposure variable. Variables with substantive differences in OR and a LR p-value ≤0.10 were assessed in the multivariable analyses for EMM. Variables that resulted in at least a 10% change in the OR were evaluated in multivariable analyses for confounding.

Adjusted prevalence OR and 95% CI for the association of interest was estimated using unconditional multiple logistic regression. We used a backward elimination strategy and evaluated variables in order of the magnitude of the Wald chi-square test p-value. Effect measure modification was evaluated by comparing the LR for the models with and without the interaction term and retaining variables with a LR p-value ≤0.10. Confounding was assessed by comparing the fully adjusted OR to the adjusted OR after removal of potentially confounding variables until the cumulative change in the OR of the exposure was >0.10.

We also performed sensitivity analyses. We assessed the robustness of the exposure definition by comparing the exposed group (OC users) to three alternative reference groups: exclusive condom users, non-OC users excluding women on other hormone-containing contraceptives and non-OC users excluding condom users. Since the relationship between vaginal lactobacilli, sex hormones and *Tv* is complex, we evaluated the impact of omitting vaginal lactobacilli from the final model. Finally, we estimated the influence an unmeasured confounder (being in a stable relationship) could have on our observed association between OC use and *Tv*

[79]. We assumed the prevalence of being in a stable relationship was 0.70 in the study population.

Results

Study population

Seven hundred ninety women were diagnosed with *Tv*. Of these, 539 (68%) infected women participated in the study and 539 (68%) were included in the present analyses. Of the 203 women negative for *Tv* recruited for the study, 177 (87%) agreed to participate.

The median age of women in this study was 27 years (standard deviation, 9 years). Most women were black (91%) and had a high school level of education or less (66%). Last year's household income was less than $12,000 for half of the women in the study. The majority of women had previously been diagnosed with an STI other than *Tv* (78%) and had more than 1 sex partner in the past year (66%). Most women did not use OC (89%), while slightly more than half reported using condoms with their most recent and/or frequent sexual partner (55%) (Table 4.1).

Bivariable Associations with T. vaginalis

Older women were more likely to have prevalent trichomoniasis compared to women 18-24 years (women 35-39 years OR=2.1, 95% CI 1.2-3.9, 40-44 years OR=3.6, 95% CI 1.6-7.7, ≥45 years OR=8.0, 95% CI 1.9-33.9) (Table 4.2). Women who were black were more likely to be infected with *Tv* compared to non-blacks

(OR=2.6, 95% CI 1.5-4.3), as were women with a lower level of education (OR=2.2, 95% CI 1.5-3.0), women with a lower household income (OR=1.8, 95% CI 1.3-2.6) and women with a lower level of vaginal lactobacilli (OR=2.2, 95% CI 1.4-3.2). Having had a past STI diagnosis, concurrent BV diagnosis, condom use and douching in the previous week were not associated with *Tv* infection status (Table 4.2).

Association between oral contraceptive use and T. vaginalis

In bivariable analyses, we observed an inverse association between OC use and prevalent *Tv* (OR=0.5, 95% CI: 0.3-0.8) (Table 4.2). Adjusting for potentially confounding covariables, including race/ethnicity, highest educational level attained, condom use, concurrent BV, level of vaginal lactobacilli, number of sex partners in the past year and number of times the woman has sex in an average week, did not substantially change the relationship (OR= 0.6, 95% CI 0.3-1.0) (Table 4.3).

Women who used OC with their most recent and/or most frequent sexual partner were considered to be exposed and women who did not use OC with their most recent and/or frequent sexual partner were classified as not being exposed. Examination of the bivariable association between OC use and *Tv* using the alternative reference groups yielded similar results: exclusive condom users (OR=0.6, 95% CI 0.3-1.0), non-OC users excluding other methods of hormonal contraceptives (OR=0.5, 95% CI 0.3-0.8), non-OC and non-condom users (OR=0.4, 95% CI: 0.2-0.7). The association between OC and *Tv* may be due to differing sexual behavior between OC users and non-users, such as being in a stable

relationship. We assessed sexual behavior with several variables, but did not have information on relationship stability. Therefore, we estimated the impact that this variable could have on our results. Assuming the prevalence of being in a stable relationship is 0.70 in the study population, the association between being in a stable relationship and OC use would have to be quite high (OR ≥5.0) to negate the observed protective effect. Finally, since vaginal lactobacilli may be in the causal pathway between OC and trichomoniasis, we excluded it from our model to assess its impact on our results. Omitting vaginal lactobacilli from the final model did not appreciably change our results (OR=0.5, 95% CI: 0.3-0.9).

Discussion

Sex hormones, such as estrogen, are known to affect STI [80]. How estrogen might effect *Tv* has not been determined. *Trichomonas vaginalis* possesses estrogen receptors, and addition of estrogen to *Tv* in culture slows growth of the organism and decreases attachment to mammalian cells [9, 10, 55]. This evidence suggests estrogen could decrease infectivity of *Tv*. However, results from studies performed in animals imply that estrogen might increase infectivity *in vivo* [9]. For example, animals that were not natural hosts for *Tv* required treatment with estrogen before establishing infection [61].

Most OC contain estrogen and are thought to influence vaginal conditions and susceptibility to infection, including chlamydia, vaginal candidiasis and BV [6-8, 30, 57, 64, 81]. While this question has been previously examined for *Tv*, study results

are variable. In this investigation, OC use was moderately protective against prevalent *Tv.*

Our findings are consistent with several other studies about the relationship between *Tv* and OC, which also found a protective effect [5, 7, 56-59]**.** However, other studies have not demonstrated a relationship between OC use and *Tv* status [6, 8, 64-66, 78]**.** This discrepancy in results may be due to the use of different diagnostic and analytical methods. For example, some studies used wet mount microscopy to diagnose *Tv* [5-7, 56-58]. Since wet mount microscopy is a less sensitive diagnostic technique compared to culture, results from these studies could be biased towards the null. Other studies employed only bivariable analyses [5, 8, 31, 57, 66]. Although we did not observe significant confounding in this study, it is possible that confounding may have influenced the results of previous studies.

The difference in *Tv* risk between OC users and non users may be due to differing sexual behaviors. However, this does not appear to be the case in this study. Our analyses included several variables related to sexual behavior (past diagnosis with an STI other than *Tv*, condom use, number of partners women had in the past year and number of times the woman has sex in an average week). Furthermore, adjustment of our final model with marital status and number of years in a sexual relationship with their most frequent partner did not substantively change our results.

The effect of OC use on cervical mucosa may protect OC users against infection [7]. However, *Tv* mainly infects the lower reproductive tract, so changes in

cervical mucosa may not have a substantial impact on trichomoniasis. In this case, changes in cervical mucosa would not explain our results.

Alternatively, female sex hormones are known to affect the reproductive tract immune response [82-85]. While the pathogenic mechanisms of *Tv* are not well understood, OC could possibly alter host defenses to *Tv*. For example, *Tv* infects vaginal epithelium, which may be thicker in women using OC compared to women not on hormonal contraception. On the other hand, OC use does not appear to affect frequency or composition of vaginal intraepithelial immune cells, so changes in vaginal epithelium due to OC use may not explain the observed results [86-89].

Finally, although it appears that estrogen decreases growth of *Tv in vitro*, it may promote growth *in vivo.* In a case report, a metronidazole-allergic postmenopausal woman was reported to have elimination of *Tv* after discontinuation of estrogen replacement therapy [63]. Establishment of an estrogenic state in hysterectomized women and animals has also been reported [63]. If estrogen is growth-promoting *in vitro*, we might expect to observe a positive association between OC and *Tv*. However, OC users appear to have low serum levels of endogenous estrogen [86]. Serum levels of endogenous estrogen were also comparatively lower among OC users versus women who did not use hormonal contraceptives [86]. An inverse association between OC and *Tv* could be explained if OC use results in a net reduction of systemic and/or local estrogen in women. An alternative explanation of the observed protective effect of OC is that estrogen from OC competes with endogenous estrogen for *Tv* estrogen receptors [7]. If endogenous estrogen promotes *Tv* growth, perhaps exogenous estrogen

competitively binds to *Tv* estrogen receptors, impeding the stimulating effect of endogenous estrogen.

In our study, we did not collect information regarding the duration of OC use. Thus, it is possible that *Tv* may have occurred before OC use, resulting in exposure misclassification. We also lacked information on OC composition. Most OC include a combination of estrogen and progesterone, though progesterone-only prescriptions exist. While the level of estrogen in different types of OC may differ, the amount is fairly constant between birth control pills [85]. Oral contraceptive misclassification should be non-differential with respect to *Tv* status and any resulting bias should be towards the null [67].

Moreover, *Tv* status may have been misclassified because of the imperfect performance of *Tv* culture. Although considered the diagnostic reference standard, *Tv* culture has imperfect sensitivity compared to *Tv* nucleic acid amplification testing. Thus, some *Tv* diagnoses may have been missed. Again, this misclassification should be non-differential with respect to OC status, and the resulting bias should lead to an underestimation of the true association.

Finally, estrogen-containing contraceptives stimulate vaginal epithelial cells to produce more glycogen, creating a favorable environment for vaginal lactobacilli [71]. If vaginal lactobacilli is in the causal pathway between OC use and *Tv*, the observed effect would be biased toward the null, and it should not be adjusted for in multivariable analyses [67]. However, exclusion of this variable from our model yielded similar results, suggesting that the effect is not mediated through this pathway.

Millions of women world-wide use contraception, and knowledge that contraceptives impact STI may affect a woman's contraceptive choice. We found OC use to be moderately protective against prevalent *Tv*. Given the popularity of OC, the high burden of *Tv* in certain populations and the variety of health problems associated with *Tv*, prospective studies are warranted to confirm these results.

Table 4.1 Characteristics of women in the study (n=716)

Characteristic		N (%)
T. vaginalis infection	yes	539 (75.28)
	no	177 (24.72)
		(n=712)
Oral contraceptive use[1]	yes	81 (11.38)
	no	631 (88.62)
		(n=407)
Endogenous estrogen (menstrual cycle days)	1-5	28 (6.88)
	6-10	83 (20.39)
	11-14	79 (19.41)
	15-25	172 (42.26)
	26-28	45 (11.06)
Age (year)	18-24	282 (39.39)
	25-29	135 (18.85)
	30-34	97 (13.55)
	35-39	89 (12.43)
	40-44	74 (10.34)
	≥45	39 (5.45)
Race/ethnicity	Black, non-Hispanic	651 (90.92)
	Other	65 (9.08)
		(n=665)
Household income, last year	<$12,000	330 (49.62)
	≥$12,000	335 (50.38)
Highest attained education	≤high school graduate or equivalent	469 (65.50)
	>high school graduate or equivalent	247 (34.50)
		(n=697)
Level of vaginal lactobacilli	0-30/field	563 (80.77)
	≥30/field	134 (19.23)
		(n=692)
Number of times has sex, average week	≥1	628 (90.75)
	0	64 (9.25)
		(n=697)

Bacterial vaginosis	yes	362 (51.94)
	no	335 (48.06)
		(n=702)
Number of times douched, past week	0 times	542 (77.21)
	1 time	129 (18.38)
	≥2 times	31 (4.42)
		(n=705)
Condom use[1]	yes	388 (55.04)
	no	317 (44.96)
		(n=713)
Past STI diagnosis[2]	no/don't know	155 (21.74)
	yes	558 (78.26)
		(n=715)
Number of sex partners, past year	1	246 (34.41)
	≥1	469 (65.59)

[1]with most recent and/or frequent sexual partner

[2]other than trichomoniasis

Table 4.2 Characteristics of women with and without *T. vaginalis* infection[1] (N=716)

Characteristic		Women with trichomoniasis (N= 539) n(%)	Women without trichomoniasis (N=177) n(%)	OR (95%CI)
Oral contraceptive use[2]	yes	50 (61.73)	31 (38.27)	0.48 (0.30-0.78)
	no	486 (77.02)	145 (22.98)	1.00
Endogenous estrogen	higher estrogen[3]	127 (39.94)	191 (60.06)	1.03 (0.63-1.66)
	lower estrogen[4]	35 (39.33)	54 (60.67)	1.00
Age (year)	18-24	197 (69.86)	85 (30.14)	1.00
	25-29	94 (69.63)	41 (30.37)	0.99 (0.63-1.55)
	30-34	71 (73.20)	26 (26.80)	1.18 (0.70-1.98)
	35-39	74 (83.15)	15 (16.85)	2.13 (1.16-3.92)
	40-44	66 (89.19)	8 (10.81)	3.56 (1.64-7.74)
	≥45	37 (94.87)	2 (5.13)	7.98 (1.88-33.87)
Race	Black, non-Hispanic	502 (77.11)	149 (22.89)	2.55 (1.51-4.31)
	Other	37 (56.92)	28 (43.08)	1.00
Education	≤high school graduate/GED	377 (80.38)	92 (19.62)	2.15 (1.52-3.04)
	>high school graduate/GED	162 (65.59)	85 (34.41)	1.00
Household income, last year	<$12,000	265 (80.30)	65 (19.70)	1.79 (1.25-2.55)
	≥$12,000	233 (69.55)	102 (30.45)	1.00
Level of vaginal lactobacilli	0-30/field	438 (77.80)	125 (22.20)	2.15 (1.44-3.22)
	≥30/field	83 (61.94)	51 (38.06)	1.00
Number of times has sex, average week	≥1	464 (73.89)	164 (26.11)	0.40 (0.19-0.87)
	0	56 (87.50)	8 (12.50)	1.00
Bacterial vaginosis	yes	271 (74.86)	91 (25.14)	1.01 (0.72-1.43)
	no	250 (74.63)	85 (25.37)	1.00
Past STI diagnosis[5]	yes	419 (75.09)	139 (24.91)	0.98 (0.65-1.48)
	no/don't know	117 (75.48)	38 (24.52)	1.00
Condom use[2]	yes	233 (73.50)	84 (26.50)	0.80 (0.57-1.13)
	no	301 (77.58)	87 (22.42)	1.00
Number of times douched, past week	0 times	138 (80.23)	34 (19.77)	1.00
	1 time	104 (80.62)	25 (19.38)	1.03 (0.58-1.82)
	≥2 times	26 (83.87)	5 (16.13)	1.28 (0.46-3.58)

[1]infection was defined as having a positive wet mount microscopy and/or *T. vaginalis* culture

[2]with most recent and/or frequent sexual partner

[3]menstrual cycle days 6-14

[4]menstrual cycle days 15-28 and 1-5

[5]other than trichomoniasis

Table 4.3 Association between oral contraceptive use *and T. vaginalis* infection

			Odds Ratio (95% CI)	
		N	Unadjusted	Adjusted
Oral contraceptive use[1]	yes	81	0.48 (0.30-0.78)	0.56 (0.32-0.97)[2]
	no	631	1.00	1.00

[1]with most recent and/or frequent sexual partner
[2]adjusted for race/ethnicity, highest educational level attained, condom use, concurrent bacterial vaginosis diagnosis, level of vaginal lactobacilli, number of sex partners, past year; number times woman has sex, average week

CHAPTER 5: DISCUSSION

Summary of Findings

Despite the fact that for many years it was considered a nuisance infection, the public health impact of trichomoniasis, the most common curable STI worldwide, is substantial [2, 11, 15]. Trichomoniasis is associated with preterm birth, low birth weight and premature rupture of the membranes [15, 90, 91]. *T. vaginalis* infection has also been implicated in the development of cervical neoplasia, tubal infertility and posthysterectomy infection [3, 14, 18]. Finally, trichomoniasis appears to increase both HIV acquisition and transmission [4, 15]. Although the prevalence in urban STD clinics is estimated to be close to 25%, trichomoniasis is not a reportable condition in the US [15].

For this dissertation, we examined the association of two aspects of the vaginal microenvironment, lactobacilli and estrogen, on prevalent trichomoniasis. We compared the prevalence of trichomoniasis in women with lower versus higher levels of vaginal lactobacilli and found that the association differed by educational level. Among women with greater than a high school education, lower levels of vaginal lactobacilli were positively associated with *Tv,* while this association was not observed in women with at most a high school education.

The effect of estrogen on trichomoniasis was assessed by estimating the association between OC use and trichomoniasis. Use of OC was found to be moderately protective against *Tv* infection.

Vaginal Lactobacilli and Trichomoniasis

Vaginal lactobacilli are the most common microorganisms in the healthy human vagina and are affected by many factors, ranging from sexual behavior to douching agents [25, 26]. Lack of vaginal lactobacilli has been positively associated with conditions such as BV, gonorrhea, HIV and chlamydia, possibly due to the production of lactic acid and H_2O_2 by vaginal lactobacilli [31-33]. *Trichomonas vaginalis* is thought to produce metabolic substances that are toxic against lactobacilli and is known to phagocytize lactobacilli, suggesting an association between low levels or absence of lactobacilli and *Tv* [92]. However, the epidemiologic data are inconsistent, and evidence for an association between vaginal lactobacilli and trichomoniasis is inconclusive.

Elucidation of the association between vaginal lactobacilli and *Tv* is worth seeking for two reasons. First, relatively little is known about the pathogenesis of trichomoniasis and factors that influence its' acquisition. Information concerning the influence of vaginal lactobacilli, the predominant vaginal microorganism in most women, on *Tv* could improve our understanding of both pathogenesis and acquisition of trichomoniasis. Secondly, restoration of vaginal microflora following cervico-vaginal conditions can affect relapse of infection, an especially pertinent issue for a condition that is often asymptomatic and may have a long duration of infectiousness [3, 46, 93]. Since exogenously applied live lactobacilli (in combination with estradiol) have been shown to help restore normal vaginal

microflora, a clearer understanding of the role of lactobacilli in the natural history of *Tv* may help refine this therapy and could possibly improve *Tv* treatment [70].

In vitro data suggest lactobacilli have a deleterious effect on *Tv*, but epidemiologic studies have produced conflicting results [34-36, 92]. In several studies trichomoniasis was associated with low levels of vaginal lactobacilli [32, 41-44]. However, in two studies that examined the association between H_2O_2 -producing lactobacilli and *Tv* infection, no statistically significant association was observed [26, 27, 31]. Another study that did not distinguish strain of *lactobacillus* failed to find an association between the presence of *Lactobacillus* spp. and *Tv* [25]. These discrepant results may be due to issues such as use of different diagnostic methods, failure to assess variables that could confound or modify the association and study population. Another factor could be the use of different comparison groups. For example, 2 of the studies that failed to find an association between vaginal lactobacilli and trichomoniasis compared H_2O_2-producing versus non- H_2O_2-producing strains of lactobacilli whereas other studies compared levels of all lactobacilli strains. Furthermore, 2 of the studies that found no association between vaginal lactobacilli and *Tv* included only 7 and 26 *Tv*-positive participants, and 1 was performed in pregnant women.

In this study, the association between vaginal lactobacilli and *Tv* infection varied by educational level. Among women with a lower level of education, *Tv* was not independently associated with low levels of vaginal lactobacilli. However, among women with greater than a high school education, low levels of vaginal lactobacilli

were positively associated with trichomoniasis, after adjusting for measured factors that could confound this relationship.

The reason for this difference is not clear and cannot be determined from the available data. Education may be a proxy for another variable. Alternatively, the observed effect modification by education could be due to chance.

Estrogen and Trichomoniasis

Sex hormones, such as estrogen, are known to affect STI. The effect of OC use on *Tv* is of particular interest because over 80 million women worldwide use OC. Oral contraceptive use affects the acquisition of other reproductive tract conditions, such as chlamydia, gonorrhea, candidiasis and BV [6, 8, 30, 50, 51, 58, 64, 77]. In order to make an informed decision about contraception, women should be informed about implications of OC on STI.

Results from previous epidemiologic studies on OC use and *Tv* are conflicting [5-8, 46, 56, 57, 65, 66, 78]. Our results are consistent with several cross-sectional studies that found an inverse association between OC use and *Tv*, although these studies used wet mount microscopy for diagnosis [5, 7, 56-58]. Another cross-sectional study used culture to diagnose *Tv* and also reported a protective effect of OC use, but this study was performed among pregnant women [59]. Finally, one prospective study reported a statistically significant inverse association between OC and trichomoniasis, although this study diagnosed *Tv* with wet mount microscopy and compared OC users to IUD users or women who had had a tubal ligation [7].

Several of these studies used multivariable analyses to adjust for potentially confounding variables [7, 56, 58, 59].

Other researchers have found no effect of OC use on *Tv* infection [6, 8, 46, 50, 64-66]. Several of these studies used wet mount microscopy to diagnose *Tv*, but most used culture. Only 1 study was prospective, but it relied on wet mount microscopy and its study population was Kenyan prostitutes [6]. Only 1 of these studies used multivariable analyses [6].

The reason for the discrepancy in results cannot be determined. Perhaps the use of different study populations, diagnostic techniques or comparison groups contributes to this phenomenon. Only 1 of the studies that failed to find a protective effect of OC use used multivariable analyses, suggesting that important covariables may need to be considered when studying this association. However, in our study, the crude OR differed only slightly from the adjusted OR, implying that confounding did not substantially influence this relationship in our study population.

While sex hormones, such as estrogen, are known to affect STI, the mechanisms by which estrogen might affect *Tv* has not been determined [80]. *Trichomonas vaginalis* possesses estrogen receptors, and addition of estrogen to *Tv* in culture slows growth of the organism and decreases attachment to mammalian cells [9, 10, 55]. Therefore, estrogen may decrease infectivity of *Tv*. However, results from *in vivo* studies suggest that estrogen might promote infectivity [9]. For example, estrogenization was required in animals who were not natural hosts of *Tv*, before *Tv* infection could be established [61, 94].

It has been suggested that an association between OC and *Tv* could be due to differences in sexual behavior between OC users and non-users [59]. This could be true, however, we adjusted for several variables related to sexual behavior.

The observed inverse association may also be explained by the effect of OC on the immune response to *Tv*, since female sex hormones are known to affect the reproductive tract immune response [82-85]. For example, women on OC may have thicker vaginal epithelia-the site of *Tv* infection- compared to women not on OC. However, OC use does not appear to impact the frequency and composition of vaginal intraepithelial immune cells [86-89].

It is also possible that systemic and/or local levels of estrogen are lower in women on OC. If estrogen promotes *Tv* growth *in vivo*, as some research has suggested, an inverse association between OC and *Tv* could be explained by a net reduction in estrogen among women on OC [63, 86]. Finally, assuming estrogen increases growth *in vivo*, estrogen from OC may compete with endogenous estrogen for *Tv* estrogen receptors [7].

Future Research Directions

The data from this study in combination with others suggest that there is an association between *Tv* and low levels of vaginal lactobacilli. To the best of our knowledge, the present study is the first to examine this relationship using culture-confirmed cases of *Tv* and multivariable analyses that considered potentially important covariables such as douching. A prospective study would help clarify the temporality of this relationship: do low levels of vaginal lactobacilli predispose

women to *Tv* acquisition or, once infected, does *Tv* lead to the destruction of vaginal lactobacilli, which leads to low levels of vaginal lactobacilli in women with trichomoniasis? Such a study should enroll non-pregnant women and have different age and racial groups well-represented. It should also diagnose *Tv* by culture or polymerase chain reaction. All participants should complete a comprehensive questionnaire including questions on demographics, sexual behavior and douching. Vaginal lactobacilli should be evaluated at baseline and regularly during follow-up. In addition to measuring the level of vaginal lactobacilli, using criteria such as that described by Nugent, it may be helpful to distinguish H_2O_2-positive and negative species [1]. While such a study would be expensive and complex, it could target all STI, to improve the understanding of these complicated relationships.

If low levels of vaginal lactobacilli do predispose women to *Tv* infection, further research could be focused on the impact of maintenance of healthy vaginal flora on *Tv* acquisition and prevention. If *Tv* leads to low levels of vaginal lactobacilli, future studies might address the potential impact screening for *Tv* could have on other conditions associated with abnormal vaginal flora, such as preterm birth.

A prospective study examining the associations between OC and *Tv* would also be helpful to further our understanding of the relationship between estrogen and *Tv*. Information on OC formulation and duration would add to the analyses, as would data on menstrual cycle regularity. Sufficient statistical power would also enable researchers, without much additional study effort, to examine the effect of progesterone-only contraceptives on *Tv*. Developing a good animal model for studying *Tv* would also be valuable, because it can be difficult to generalize *in vitro*

data to humans. Studies on the effect of OC on circulating and local levels of estrogen and how it influences the vaginal immune response would also be useful.

Given the prevalence of *Tv* worldwide, research concerning the public health impact of *Tv* screening, particularly in populations with a high prevalence of *Tv* and HIV should be a high priority. If additional studies substantiate the protective effect of OC against *Tv*, it may be helpful to examine the potential role OC could play in the HIV epidemic. This topic is complicated since OC has been associated with an increased prevalence of other STI. Finally, public health policy must be changed to ensure *Tv* is reportable nationally. Until then, the true burden of trichomoniasis in the US will not be known.

In summary, my dissertation examined the effect of the vaginal microenvironment on *Tv* infection. Both low levels of vaginal lactobacilli and OC use were associated with trichomoniasis, though in different directions. The temporality of the relationship between vaginal lactobacilli and *Tv* remains to be elucidated, and could have implications for efforts for vaginal flora restoration and/or *Tv* screening. The mechanism by which OC protects against *Tv* also needs to be clarified. This association may impact women's contraceptive choice and could possibly play a role in the HIV epidemic.

References

1. Nugent RP, Krohn MA and Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. J Clin Microbiol 1991;29:297-301

2. WHO. Global prevalence and incidence of selected curable sexually transmitted infections, 2001

3. Soper D. Trichomoniasis: Under control or undercontrolled? Am J Obstet Gynecol 2004;190:281-290

4. Laga M, Manoka A, Kivuvu M, et al. Non-ulcerative sexually transmitted diseases as risk factors for HIV-1 transmission in women: results from a cohort study. Aids 1993;7:95-102

5. Bramley M, Kinghorn G. Do oral contraceptives inhibit Trichomonas vaginalis? Sex Transm Dis 1979;6:261-3

6. Baeten JM, Nyange PM, Richardson BA, et al. Hormonal contraception and risk of sexually transmitted disease acquisition: results from a prospective study. Am J Obstet Gynecol 2001;185:380-5

7. Barbone F, Austin H, Louv WC and Alexander WJ. A follow-up study of methods of contraception, sexual activity, and rates of trichomoniasis, candidiasis, and bacterial vaginosis. Am J Obstet Gynecol 1990;163:510-4

8. Ceruti M, Canestrelli M, Condemi V, et al. Methods of contraception and rates of genital infections. Clin Exp Obstet Gynecol 1994;21:119-23

9. Sugarman B, Mummaw N. Oestrogen binding by and effect of estrogen on trichomonads and bacteria. J Med Microbiol 1990;32:227-232

10. Ford LC, Hammill HA, DeLange RJ, et al. Determination of estrogen and androgen receptors in Trichomonas vaginalis and the effects of antihormones. Am J Obstet Gynecol 1987;156:1119-21

11. Rein M. Trichomonas vaginalis and Trichomoniasis. In: KK H, ed. Sexually Transmitted Diseases. New York: McGraw-Hill, 1990:481-492

12. Moodley P, Wilkinson D, Connolly C, Moodley J and Sturm AW. Trichomonas vaginalis is associated with pelvic inflammatory disease in women infected with human immunodeficiency virus. Clin Infect Dis 2002;34:519-22

13. El-Shazly AM, El-Naggar HM, Soliman M, et al. A study on Trichomoniasis vaginalis and female infertility. J Egypt Soc Parasitol 2001;31:545-53

14. Soper DE, Bump RC and Hurt WG. Bacterial vaginosis and trichomoniasis vaginitis are risk factors for cuff cellulitis after abdominal hysterectomy. Am J Obstet Gynecol 1990;163:1016-21; discussion 1021-3

15. Schwebke JR, Burgess D. Trichomoniasis. Clin Microbiol Rev 2004;17:794-803.

16. Cotch MF, Pastorek JG, 2nd, Nugent RP, et al. Trichomonas vaginalis associated with low birth weight and preterm delivery. The Vaginal Infections and Prematurity Study Group. Sex Transm Dis 1997;24:353-60

17. Yap EH, Ho TH, Chan YC, et al. Serum antibodies to Trichomonas vaginalis in invasive cervical cancer patients. Genitourin Med 1995;71:402-4

18. Zhang ZF, Graham S, Yu SZ, et al. Trichomonas vaginalis and cervical cancer. A prospective study in China. Ann Epidemiol 1995;5:325-32

19. Sorvillo F, Smith L, Kerndt P and Ash L. Trichomonas vaginalis, HIV, and African-Americans. Emerg Infect Dis 2001;7:927-32

20. Sorvillo F, Kerndt P. Trichomonas vaginalis and amplification of HIV-1 transmission. Lancet 1998;351:213-4

21. ter Meulen J, Mgaya HN, Chang-Claude J, et al. Risk factors for HIV infection in gynaecological inpatients in Dar es Salaam, Tanzania, 1988-1990. East Afr Med J 1992;69:688-92

22. HIV/AIDS WHODo. Global prevalence and incidence of selected curable sexually transmitted infections. Vol. 2005, 2001

23. Cates W. Estimates of the incidence and prevalence of sexually transmitted diseases in the United States. Sex Transm Dis 1999;26:S2-S7

24. Weinstock H, Berman S and Cates W, Jr. Sexually transmitted diseases among American youth: incidence and prevalence estimates, 2000. Perspect Sex Reprod Health 2004;36:6-10

25. Newton ER, Piper JM, Shain RN, Perdue ST and Peairs W. Predictors of the vaginal microflora. Am J Obstet Gynecol 2001;184:845-53

26. Martin HL, Richardson BA, Nyange PM, et al. Vaginal lactobacilli, microbial flora, and risk of human immunodeficiency virus type 1 and sexually transmitted disease acquisition. J Infect Dis 1999;180:1863-8

27. Hawes SE, Hillier SL, Benedetti J, et al. Hydrogen peroxide-producing lactobacilli and acquisition of vaginal infections. J Infect Dis 1996;174:1058-63

28. Eschenbach DA, Davick PR, Williams BL, et al. Prevalence of hydrogen peroxide-producing Lactobacillus species in normal women and women with bacterial vaginosis. J Clin Microbiol 1989;27:251-6

29. Boskey ER, Telsch KM, Whaley KJ, Moench TR and Cone RA. Acid production by vaginal flora in vitro is consistent with the rate and extent of vaginal acidification. Infect Immun 1999;67:5170-5

30. McGregor J, Hammill H. Contraception and sexually transmitted diseases: interactions and opportunities. Am J Obstet Gynecol 1993;168:2033-2041

31. Hillier S, Krohn M, Klebanoff S and Eschenbach D. The relationship of hydrogen peroxide-producing lactobacilli to bacterial vaginosis and genital microflora in pregnant women. Obstet Gynecol 1992;79:369-373

32. Hillier SL, Krohn MA, Nugent RP and Gibbs RS. Characteristics of three vaginal flora patterns assessed by gram stain among pregnant women. Vaginal Infections and Prematurity Study Group. Am J Obstet Gynecol 1992;166:938-44

33. Sewankambo N, Gray RH, Wawer MJ, et al. HIV-1 infection associated with abnormal vaginal flora morphology and bacterial vaginosis. Lancet 1997;350:546-50

34. McGrory T, Meysick K, Lemchuk-Favel LT and Garber GE. The interaction of Lactobacillus acidophilus and Trichomonas vaginalis in vitro. J Parasitol 1994;80:50-4

35. Rendon-Maldonado JG, Espinosa-Cantellano M, Gonzalez-Robles A and Martinez-Palomo A. Trichomonas vaginalis: in vitro phagocytosis of lactobacilli, vaginal epithelial cells, leukocytes, and erythrocytes. Exp Parasitol 1998;89:241-50

36. Juliano C, Cappuccinelli P and Mattana A. In vitro phagocytic interaction between Trichomonas vaginalis isolates and bacteria. Eur J Clin Microbiol Infect Dis 1991;10:497-502

37. Butler B, Beakley J. Bacterial flora in vaginitis: a study before and after treatment with pure cultures of Doderlein bacillus. Am J Obstet Gynecol 1960;79:432-440

38. Sprott MS, Kearns AM and Pattman RS. Trichomonal vaginitis refractory to treatment: case report. Genitourin Med 1988;64:369-72

39. Parsons CL, Lofland S and Mulholland SG. The effect of trichomonal vaginitis on vaginal pH. J Urol 1977;118:621-2

40. Robinson SC, Mirchandani G. Observations On Vaginal Trichomoniasis. Iv. Significance Of Vaginal Flora Under Various Conditions. Am J Obstet Gynecol 1965;91:1005-12

41. Donders G, De Wet HG, Hooft P and Desmyter J. Lactobacilli in Papanicolaou smears, genital infections, and pregnancy. Am J Perinatol 1993;10:358-61

42. Moodley P, Connolly C and Sturm AW. Interrelationships among human immunodeficiency virus type I infection, bacterial vaginosis, trichomoniasis, and the presence of yeasts. JID 2002;185:69-73

43. Donders GG, Bosmans E, Dekeersmaecker A, Vereecken A, Van Bulck B and Spitz B. Pathogenesis of abnormal vaginal bacterial flora. Am J Obstet Gynecol 2000;182:872-8

44. James JA, Thomason JL, Gelbart SM, Osypowski P, Kaiser P and Hanson L. Is trichomoniasis often associated with bacterial vaginosis in pregnant adolescents? Am J Obstet Gynecol 1992;166:859-63

45. Hanna N, Taylor-Robinson D, Kalodiki-Karamanoli M and McFadyen I. The relation between vaginal pH and the microbiological status in vaginitis. British journal of obstetrics and gynecology 1985;92:1267-1271

46. Fouts AC, Kraus SJ. Trichomonas vaginalis: reevaluation of its clinical presentation and laboratory diagnosis. J Infect Dis 1980;141:137-143

47. Schwebke JR, Richey CM and Weiss HL. Correlation of behaviors with microbiological changes in vaginal flora. J Infect Dis 1999;180:1632-6

48. Stevens-Simon C, Jamison J, McGregor JA and Douglas JM. Racial variation in vaginal pH among healthy sexually active adolescents. Sex Transm Dis 1994;21:168-72

49. Swygard H, Sena A, Hobbs M and Cohen M. Trichomoniasis:clinical manifestations diagnosis and management. Sex Transm Infect 2004;80:91-95

50. Avonts D, Sercu M, Heyerick P, Vandermeeren I, Meheus A and Piot P. Incidence of uncomplicated genital infections in women using oral contraception or an intrauterine device: a prospective study. Sex Transm Dis 1990;17:23-29

51. Moi H. Prevalence of bacterial vaginosis and its association with genital infections, inflammation, and contraceptive methods in women attending sexually transmitted disease and primary health clinics. Int J STD AIDS 1990;1:86-94

52. Heine P, McGregor JA. Trichomonas vaginalis: a reemerging pathogen. Clin Obstet Gynecol 1993;36:137-44

53. Eckert LO, Hawes SE, Stevens CE, Koutsky LA, Eschenbach DA and Holmes KK. Vulvovaginal candidiasis: clinical manifestations, risk factors, management algorithm. Obstet Gynecol 1998;92:757-65

54. Manhart LE, Critchlow CW, Holmes KK, et al. Mucopurulent cervicitis and Mycoplasma genitalium. J Infect Dis 2003;187:650-7

55. Sugarman B, Mummaw N. The effect of hormones on *Trichomonas vaginalis*. Journal of General Microbiology 1988;134:1623-1628

56. Pereira LH, Embil JA, Haase DA and Manley KM. Cytomegalovirus infection among women attending a sexually transmitted disease clinic: association with clinical symptoms and other sexually transmitted diseases. Am J Epidemiol 1990;131:683-92

57. Kinghorn GR, Waugh MA. Oral contraceptive use and prevalence of infection with Chlamydia trachomatis in women. Br J Vener Dis 1981;57:187-90

58. Gertig DM, Kapiga SH, Shao JF and Hunter DJ. Risk factors for sexually transmitted diseases among women attending family planning clinics in Dar-es-Salaam, Tanzania. Genitourin Med 1997;73:39-43

59. Cotch M, Pastorek J, Nugent R, Yerg D, Martin D and Eschenbach D. Demographic and behavioral predictors of *Trichomonas vaginalis* infection among pregnant women. Obstetrics & Gynecology 1991;78:1087-1092

60. Garber GE, Lemchuk-Favel LT and Rousseau G. Effect of beta-estradiol on production of the cell-detaching factor of Trichomonas vaginalis. J Clin Microbiol 1991;29:1847-9

61. Sonnex C. Influence of ovarian hormones on urogenital infection. Sex Transm Inf 1998;74:11-19

62. Meysick KC, Garber GE. Interactions between Trichomonas vaginalis and vaginal flora in a mouse model. J Parasitol 1992;78:157-60

63. Sharma R, Pickering J and Mccormack W. Trichomoniasis in a postmenopausal women cured after discontinuation of estrogen replacement therapy. Sex Transm Dis 1997;24:543-545

64. Oh MK, Feinstein RA, Soileau EJ, Cloud GA and Pass RF. Chlamydia trachomatis cervical infection and oral contraceptive use among adolescent girls. J Adolesc Health Care 1989;10:376-81

65. Lefevre JC, Averous S, Bauriaud R, Blanc C, Bertrand MA and Lareng MB. Lower genital tract infections in women: comparison of clinical and epidemiologic findings with microbiology. Sex Transm Dis 1988;15:110-3

66. Staerfelt F, Gundersen TJ, Halsos AM, et al. A survey of genital infections in patients attending a clinic for sexually transmitted diseases. Scand J Infect Dis Suppl 1983;40:53-7

67. Rothman K, Greenland S. Modern Epidemiology. 2nd ed.: Philadelphia: Lippincott Williams & Wilkins, 1998

68. Cohen L. Influence of pH on vaginal discharges. Br J Vener Dis 1969;45:241-7

69. Bowden F, Garnett G. Trichomonas vaginalis epidemiology: parameterising and analysing a model of treatment intervenions. Sex Transm Infect 2000;76:248-256

70. Ozkinay E, Terek MC, Yayci M, Kaiser R, Grob P and Tuncay G. The effectiveness of live lactobacilli in combination with low dose oestriol (Gynoflor) to restore the vaginal flora after treatment of vaginal infections. Bjog 2005;112:234-40

71. Paavonen J. Physiology and ecology of the vagina. Scand J Infect Dis Suppl 1983;40:31-5

72. Onderdonk A, Delaney M, Hinkson P and DuBois A. Quantitative and qualitative effects of douche preparations on vaginal microflora. Obstet Gynecol 1992;80:333-338

73. Scholes D, Stergachis A, Ichikawa LE, Heidrich FE, Holmes KK and Stamm WE. Vaginal douching as a risk factor for cervical Chlamydia trachomatis infection. Obstet Gynecol 1998;91:993-7

74. Josoef M, Sumampouw H, Linnan M, Schmid S, Idajadi A and St.Louis M. Douching and sexually transmitted diseases in pregnant women in Surabaya, Indonesia. Am J Obstet Gynecol 1996;174:115-119

75. Ness R, Hillier S, Richter H, et al. Douching in relation to bacterial vaginosis, lactobacilli, and facultative bacteria in the vagina. Obstet Gynecol 2002;100:765-772

76. CDC. Tracking the Hidden Epidemics 2000: Trends in STDs in the United States. Vol. 2005, 2004

77. UN. World Contraceptive Use 2005. Vol. 2006, 2005

78. Mohllajee AP, Curtis KM, Martins SL and Peterson HB. Hormonal contraceptive use and risk of sexually transmitted infections: a systematic review. Contraception 2006;73:154-65

79. Flanders WD, Khoury MJ. Indirect assessment of confounding: graphic description and limits on effect of adjusting for covariates. Epidemiology 1990;1:239-46

80. Brabin L. Hormonal markers of susceptibility to sexually transmitted infections: are we taking them seriously? Bmj 2001;323:394-5

81. Cottingham J, Hunter D. Chlamydia trachomatis and oral contraceptive use: a quantitative review. Genitourin Med 1992;68:209-16

82. Prakash M, Patterson S, Gotch F and Kapembwa MS. Ex vivo analysis of HIV-1 co-receptors at the endocervical mucosa of women using oral contraceptives. Bjog 2004;111:1468-70

83. Beagley KW, Gockel CM. Regulation of innate and adaptive immunity by the female sex hormones oestradiol and progesterone. FEMS Immunol Med Microbiol 2003;38:13-22

84. White HD, Crassi KM, Givan AL, et al. CD3+ CD8+ CTL activity within the human female reproductive tract: influence of stage of the menstrual cycle and menopause. J Immunol 1997;158:3017-27

85. Ghanem KG, Shah N, Klein RS, et al. Influence of sex hormones, HIV status, and concomitant sexually transmitted infection on cervicovaginal inflammation. J Infect Dis 2005;191:358-66

86. Ildgruben A, Sjoberg I, Hammarstrom ML and Backstrom T. Steroid receptor expression in vaginal epithelium of healthy fertile women and influences of hormonal contraceptive usage. Contraception 2005;72:383-92

87. Ildgruben AK, Sjoberg IM and Hammarstrom ML. Influence of hormonal contraceptives on the immune cells and thickness of human vaginal epithelium. Obstet Gynecol 2003;102:571-82

88. Eschenbach DA, Patton DL, Meier A, et al. Effects of oral contraceptive pill use on vaginal flora and vaginal epithelium. Contraception 2000;62:107-12

89. Patton DL, Thwin SS, Meier A, Hooton TM, Stapleton AE and Eschenbach DA. Epithelial cell layer thickness and immune cell populations in the normal human

vagina at different stages of the menstrual cycle. Am J Obstet Gynecol 2000;183:967-73

90. Minkoff H, Grunebaum AN, Schwarz RH, et al. Risk factors for prematurity and premature rupture of membranes: a prospective study of the vaginal flora in pregnancy. Am J Obstet Gynecol 1984;150:965-72

91. Hardy PH, Hardy JB, Nell EE, Graham DA, Spence MR and Rosenbaum RC. Prevalence of six sexually transmitted disease agents among pregnant inner-city adolescents and pregnancy outcome. Lancet 1984;2:333-7

92. Petrin D, Delgaty K, Bhatt R and Garber G. Clinical and microbiological aspects of Trichomonas vaginalis. Clin Microbiol Rev 1998;11:300-17

93. Mbizvo EM, Msuya SE, Stray-Pedersen B, Sundby J, Chirenje ZM and Hussain A. Determinants of reproductive tract infections among asymptomatic women in Harare, Zimbabwe. Cent Afr J Med 2001;47:57-64

94. McGrory T, Garber GE. Mouse intravaginal infection with Trichomonas vaginalis and role of Lactobacillus acidophilus in sustaining infection. Infect Immun 1992;60:2375-9