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Association of *Chlamydia trachomatis* with Persistence of High-Risk Types of Human Papillomavirus in a Cohort of Female Adolescents

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Human papillomavirus (HPV) infection is a necessary but not sufficient cause of cervical cancer. While chlamydia infection has been associated with cervical cancer, the meaning of this association remains unclear. The authors' objective was to investigate this association by evaluating whether concurrent genital tract infections are associated with HPV persistence, a precursor to cervical cancer. Interview data and biologic samples for HPV, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, and bacterial vaginosis testing were collected from female adolescents in an Atlanta, Georgia, longitudinal cohort study at 6-month visits (1999–2003). Associations with persistence (detection of the same HPV type at two sequential visits (visit pair)) were assessed among subjects with 2–5 visits and $\geq$6 months of follow-up. Associations were evaluated by logistic regression using methods for correlated data. Type-specific persistence of high-risk HPV types was detected in 77 of 181 (43%) analyzed visit pairs. Concurrent infection with *C. trachomatis* was independently associated with persistence of high-risk HPV types (adjusted odds ratio = 2.1, 95% confidence interval: 1.0, 4.1). Infection with more than one HPV type at the initial visit was also associated with high-risk persistence (adjusted odds ratio = 2.8, 95% confidence interval: 1.6, 4.9). The association between chlamydia infection and cervical cancer may be due to an effect of chlamydia infection on persistence of high-risk HPV.

adolescent; *Chlamydia trachomatis*; *Neisseria gonorrhoeae*; longitudinal studies; papillomavirus, human; sexually transmitted diseases; *Trichomonas vaginalis*; vaginosis, bacterial

Abbreviations: CI, confidence interval; HPV, human papillomavirus; OR, odds ratio.

Human papillomavirus (HPV) infection is a necessary but not sufficient cause of cervical cancer. Most HPV infections are resolved by the host immune response, and it is thought that host or external cofactors are required for progression to cancer. Studies examining associations between other sexually transmitted infections and cervical cancer (1–5) have demonstrated associations between cervical cancer and *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, and herpes simplex virus type 2 in analyses controlling for HPV infection status (1, 4), which suggests that these sexually transmitted infections may be cofactors for the development of cancerous lesions.

The association between a history of sexually transmitted infection and cervical cancer has been explored most thoroughly for *C. trachomatis*. Case-control studies controlling for HPV infection status have demonstrated an association between detection of antibodies to *C. trachomatis* and the development of cervical cancer (1, 2, 6, 7), providing supporting evidence for this hypothesis. A retrospective study demonstrated an increased risk of cervical cancer in women

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who had a history of *C. trachomatis* infection (7). These studies showed an association between *C. trachomatis* infection and subsequent cervical cancer but were unable to evaluate the role of concurrent *C. trachomatis* and HPV infections, either because the concurrency of these infections could not be determined or because few participants with concurrent infection were present in the study populations.

It is possible that changes in the host response to HPV that occur during concurrent infection may decrease the host's ability to resolve the HPV infection. If present, this effect would be detected as increased persistence of HPV infection. Persistence of HPV infection has been closely associated with progression to cancer (8–10). Therefore, detection of an association between concurrent *C. trachomatis* infection and increased HPV persistence would support the association between *C. trachomatis* infection and cervical cancer and would suggest a mechanism for this association. To evaluate the effect of concurrent infection on HPV infection, we analyzed data from a prospective longitudinal cohort study. We examined associations between concurrent *C. trachomatis* infection and other genital tract infections and type-specific HPV persistence.

MATERIALS AND METHODS

Study population

Study participants were recruited from a primary care clinic at a public pediatric hospital in Atlanta, Georgia. All sexually active females aged 13–19 years were eligible to participate if a pelvic examination was indicated. Participants were excluded if they were pregnant or infected with human immunodeficiency virus or if they had been treated with antibiotics in the past month. For adolescents aged 13–17 years, the informed assent of the adolescent and the permission of a parent/guardian were obtained prior to enrollment; for adolescents aged 18 or 19 years, informed consent was obtained prior to enrollment. The study protocol was reviewed and approved by the institutional review boards at the Centers for Disease Control and Prevention and Emory University. The human experimentation guidelines of the US Department of Health and Human Services and Emory University were followed.

In this analysis, we evaluated study data collected between January 1999 and May 2003. During that period, 621 girls were enrolled. Data for girls not enrolled in the study were not available.

Data collection

A questionnaire was administered in private by trained interviewers as previously described (11). The questionnaire included questions on demographic factors, sexual and reproductive history, condom use, and drug and alcohol use.

Papanicolaou testing and HPV detection

At the time of pelvic examination, cervical cells from the endo- and ectocervix were collected using the Cytyc plastic broom collection kit and placed in 20 ml of PreservCyt cytologic fixative following the manufacturer's protocol for a routine diagnostic ThinPrep Papanicolaou smear (Cytyc Corporation, Marlborough, Massachusetts); this procedure was performed at every visit. ThinPrep Papanicolaou smears were prepared and evaluated in the hospital cytopathology laboratory. The cytologic finding, based on the Bethesda classification (12), was recorded from the resulting clinical report.

Residual PreservCyt cervical material was retrieved from the cytology laboratory. A 3-ml aliquot of resuspended cells was washed with 5 ml of Dulbecco's phosphate-buffered saline (Gibco BRL, Gaithersburg, Maryland) and extracted with the MasterPure total nucleic acid extraction kit (Epicentre, Madison, Wisconsin) using minor modifications to the manufacturer's protocol as previously described (11). One blank tube was included for every 10 samples to monitor cross-sample contamination.

HPV detection and typing was performed using the Roche line blot assay (Roche Molecular Systems, Inc., Pleasanton, California) as previously described (11). This assay uses HPV L1 consensus polymerase chain reaction with biotinylated PGMY09/11 primer sets and β-globin as an internal control for sample amplification (13, 14). Total nucleic acid from CaSki (cervical carcinoma) cells harboring HPV 16 was used as the positive control. Amplicons (10 μl) were evaluated for β-globin and HPV bands using 1.5 percent agarose gel electrophoresis stained with ethidium bromide, and those with an HPV band were hybridized to the typing strips. Samples with an HPV band that did not hybridize to the strip were sequenced for determination of HPV type as previously described (15). Samples negative for β-globin and HPV were considered inadequate for interpretation.

Specimen collection and detection of concurrent infections

Specimens were collected for detection of *C. trachomatis*, *N. gonorrhoeae*, *Trichomonas vaginalis*, and bacterial vaginosis at every study visit. First-catch urine specimens were collected for *C. trachomatis* and *N. gonorrhoea* testing for all study participants, using ligase chain reaction (LCx; Abbott Laboratories, Abbott Park, Illinois), polymerase chain reaction (COBAS AMPLICOR; Roche Diagnostic Systems, Branchburg, New Jersey), or strand displacement amplification (BD ProbeTec; BD Biosciences, Sparks, Maryland), according to manufacturers' instructions. Because of changing testing protocols, a cervical swab was also collected for some participants and tested for *C. trachomatis* and *N. gonorrhoea* with ligase chain reaction or strand displacement amplification.

In 355 visits where results of urine and cervical *C. trachomatis* testing were available, discrepant results were found for 12 visits (at eight visits the urine specimen only was positive; at four the cervical specimen only was positive). In 354 visits with results from urine and cervical *N. gonorrhoea* testing, discrepant results were found for eight visits (at seven the urine specimen only was positive; at one the cervical specimen only was positive). Any

positive *C. trachomatis* or *N. gonorrhoea* test was considered a positive result.

Lateral-wall vaginal swabs were collected for *T. vaginalis* and bacterial vaginosis testing. *T. vaginalis* infection was detected through wet mount examination of vaginal fluid under microscopy and was confirmed by at least one other reader (E. H. K. or M. K. S.). For detection of bacterial vaginosis, vaginal swabs were rolled onto clean glass slides, air-dried, Gram-stained, and read (E. H. K.) using Nugent's criteria (16); a Nugent's score of 7–10 was considered evidence of bacterial vaginosis. Samples for bacterial vaginosis analysis were not collected during the first year of the study.

Study participants with *C. trachomatis*, *N. gonorrhoea*, *T. vaginalis*, or bacterial vaginosis were treated with appropriate directly observed one-dose therapy. Cure of *C. trachomatis* and *N. gonorrhoea* infections was evaluated by urine testing, and no treatment failures were detected.

Data analysis

To evaluate factors associated with HPV persistence, we limited our analysis to pairs of visits where 1) HPV was detected at the initial visit of the pair, 2) HPV detection data were available for a subsequent visit, and 3) the visits were separated by at least 6 months. Any visit pair meeting these criteria was included in the analysis. More than one visit pair could be contributed by one study participant. Therefore, a study participant with three study visits separated by 6 months and detection of HPV 18 and HPV 53 at visits 1 and 2 and detection of HPV 26 at visit 2 would contribute two visit pairs (visits 1–2 and visits 2–3). A type-specific persistent outcome occurred when at least one of the HPV types detected at the initial visit of the pair was also detected at the second visit; if none of the types present at the initial visit were detected at the second visit, the outcome was considered nonpersistent (17). Therefore, the study participant described above would contribute one outcome of 6-month persistence for each of HPV 18 and HPV 53 and one nonpersistent outcome for HPV 26.

One negative test was considered a marker of clearance. Redetection of an HPV type after a single negative result (a sequence of successive tests with positive–negative–positive findings, suggesting a potential false-negative result) occurred rarely (nine times) in these data. Omission of these events did not alter the results of the analysis.

After type-specific assessment of persistence, outcomes were grouped into high-risk (types 16, 18, 26, 31, 33, 35, 39, 45, 51–53, 55, 56, 58, 59, 66, 68, 73, 82, and 83) and low-risk (types 6, 11, 40, 42, 54, 57, and 84) HPV types, according to the association between type and oncogenicity. If persistence of any high-risk type occurred over the period of a visit pair, one episode of high-risk persistence was generated; if no high-risk infection persisted, one outcome of nonpersistent high-risk infection was generated. Therefore, the example study participant described above would contribute one outcome of high-risk persistence over visits 1–2 and one outcome of nonpersistence over visits 2–3. These criteria were also applied to detection of low-risk virus types. If both high-risk and low-risk types were detected at the initial visit of a pair, one high-risk episode and one low-risk episode were contributed. Since persistence of more than one HPV type over a given pair of visits was not uncommon, use of one outcome per risk type per visit pair is conservative. Clearance of two virus types over a single pair of visits may reflect the same immunologic event; therefore, counting this event twice may inappropriately magnify the effect seen.

Logistic regression using generalized estimating equations with an exchangeable correlation structure, a method appropriate for correlated data (18), was used to evaluate associations between type-specific high- or low-risk persistence and concurrent infections (*C. trachomatis*, *N. gonorrhoea*, *T. vaginalis*, bacterial vaginosis, or more than one type of HPV) detected at the initial visit of the visit pair and to assess the role of potential confounders or effect modifiers (lifetime and current numbers of sex acts or sex partners, time between study visits, age, smoking, oral contraceptive use, parity, and douching) (17, 19–22). Univariate analyses were used to assess two-way associations; variables significant in univariate analysis were entered into a multivariate model, and determination of significance was based on robust standard errors.

RESULTS

Study population characteristics

Study participants for whom 2–5 study visits 6 months apart had been completed (282 participants contributing 335 study visits) were evaluated. The cumulative prevalence of HPV in these participants was 78 percent (219/282). Among these 282 participants, HPV was detected at the initial visit of a visit pair in 151. This group, contributing 181 visit pairs, was our final study population. These 151 participants did not differ from the study participants not included in this analysis with regard to age, race, ethnicity, age at first sex, number of sex partners, or *C. trachomatis* prevalence at baseline.

Behavioral and demographic characteristics of this population are presented in table 1. The majority of the analyzed participants were African-American and in high school, with a median age at first sex of 14 years. The prevalence of all sexually transmitted infections evaluated in this population was high. Concurrent infection with more than one of these sexually transmitted agents was not uncommon; infection with both *C. trachomatis* and *N. gonorrhoea* or *C. trachomatis* and *T. vaginalis* was detected in 26 (17 percent) of the 151 study participants contributing analyzable visits.

HPV persistence

Data on the persistence of HPV by type are presented in table 2. Some study participants were infected with more than one type of HPV. Therefore, summation of persistence by HPV type (as shown in table 2) generates a larger number of outcomes than the persistence analysis reported in the text, which grouped high-risk types and used only one outcome per visit pair.

TABLE 1. Demographic and behavioral characteristics of female adolescent study participants ($n = 151$) from a primary care clinic in whom human papillomavirus was detected at the initial visit of a visit pair, Atlanta, Georgia, 1999–2003

Characteristic	No.	%
Median age (years)	16 (13–19)*	
Race/ethnicity		
African-American	143	95
Other	7	5
No information	1	0
Attending secondary school		
Yes	113	75
No	21	14
No information	17	11
Median age (years) at first sex	14 (11–17)	
Median lifetime no. of sex partners at baseline	3 (1–150)	

* Numbers in parentheses, range.

TABLE 2. Six-month persistence of human papillomavirus (HPV) by HPV type among analyzed visit pairs contributed by female adolescent study participants, Atlanta, Georgia, 1999–2003*

HPV type	No. of participants	Type-specific persistence	
		No.	%
Low-risk type			
HPV6	23	3	13
HPV11	3	1	33
HPV40	9	0	0
HPV42	11	1	9
HPV54	13	4	31
HPV57	1	0	0
HPV84	23	5	22
Any low-risk type	71	13	17
High-risk type			
HPV16	45	17	38
HPV18	20	6	30
HPV26	4	1	25
HPV31	11	4	45
HVP33	5	0	0
HPV35	22	9	41
HPV39	17	4	24
HPV45	14	6	43
HPV51	19	2	11
HPV52	23	8	35
HPV53	15	4	27
HPV55	8	0	0
HPV56	11	5	46
HPV58	24	10	42
HPV59	28	5	18
HPV66	24	9	38
HPV68	21	7	33
HPV73	14	4	29
HPV82	9	0	0
HPV83	10	4	40
Any high-risk type	181	77	43

* Because more than one type of HPV may have been present at a given visit, summation by HPV type will not equal the totals given for "Any low-/high-risk type."

A larger proportion of high-risk infections persisted than low-risk infections. Of 181 visit pairs with a high-risk HPV type detected at the first visit of the pair, the same high-risk type was detected at the second visit of the pair in 77 (43 percent). Of 71 visit pairs with a low-risk virus type detected at the first visit, the same virus type was detected at the second visit in 13 (17 percent).

We evaluated whether HPV persistence was associated with abnormal cytologic findings, as has been reported for other populations (10, 23). Persistence was associated with detection of low- or high-grade squamous intraepithelial lesions ($p < 0.01$); 19 (59 percent) of 32 low- or high-grade squamous intraepithelial lesions identified were detected at a visit at which high-risk HPV persistence was also detected (i.e., the second visit of a visit pair). Only four of these lesions were high-grade squamous intraepithelial lesions.

Risk factor analysis

Data on the detection and persistence of high- and low-risk HPV types and the proportions of participants coinfected with *C. trachomatis*, *N. gonorrhoea*, *T. vaginalis*, bacterial vaginosis, or another HPV type at the initial visit of the visit pair are shown in table 3. A higher proportion of high-risk HPV types persisted if any evaluated sexually transmitted infection was present at the initial visit of the pair; for example, among high-risk infections at visits with concurrent *C. trachomatis* infection, 53 percent persisted, while 39 percent of infections at visits with no concurrent *C. trachomatis* infection persisted. This pattern was not seen for low-risk HPV types.

To evaluate associations between concurrent infections detected at the initial visit and persistence of HPV to the following visit, we performed univariate and multivariate logistic regression analyses appropriate for correlated data. No significant associations were found with persistence of low-risk infections (data not shown). Table 4 shows univariate and multivariate associations between high-risk HPV persistence and concurrent infections. In univariate analysis, concurrent infection with *C. trachomatis* (odds ratio (OR) = 1.9, 95 percent confidence interval (CI): 1.0, 3.6) or another HPV type (OR = 2.6, 95 percent CI: 1.5, 4.9) at the initial visit was associated with persistence of high-risk HPV infections to the following visit. No other concurrent infections of interest were significantly associated with persistence, nor was detection of *C. trachomatis*

TABLE 3. Detection and persistence of high- and low-risk types of human papillomavirus (HPV) and proportions of participants with concurrent infection among analyzed visit pairs contributed by female adolescent study participants, Atlanta, Georgia, 1999–2003

Concurrent infection at first visit of visit pair	High-risk HPV type			Low-risk HPV type		
	No. of infections detected	Persistent infections		No. of infections detected	Persistent infections	
		No.	%		No.	%
Chlamydia trachomatis						
Present	47	25	53	18	4	22
Absent	134	51	39	53	9	17
*Neisseria gonorrhoeae**						
Present	23	11	48	13	1	8
Absent	158	66	42	57	11	19
Trichomonas vaginalis						
Present	13	7	54	5	0	0
Absent	168	70	42	66	13	20
Bacterial vaginosis						
Present	53	24	45	23	5	22
Absent	62	23	37	23	5	22
Not tested	66	30	46	25	3	12
More than one type of HPV						
Present	88	48	55	36	8	22
Absent	93	29	31	35	5	14
Total	181	77	43	71	13	18

* For analysis of visit pairs with low-risk HPV infection, the total number of infections detected was 70.

at the second visit of the visit pair (27 of 181 visit pairs; 15 percent). In a multivariate model, concurrent infection with *C. trachomatis* and concurrent infection with another type of HPV were each independently associated with persistence of a high-risk infection (for *C. trachomatis*, OR = 2.1, 95 percent CI: 1.0, 4.1; for more than one HPV type, OR = 2.8, 95 percent CI: 1.6, 4.9). No interaction was detected. Neither concurrent infection with *C. trachomatis* nor concurrent infection with another HPV type was associated with detection of low- or high-grade squamous intraepithelial lesions.

To evaluate whether detection of the same HPV type at two sequential study visits reflected reinfection, we assessed whether there was an association between the number of sex partners or the number of sex acts reported in the 90 days prior to the second visit of the pair and HPV persistence. No association was detected between these factors and persistence. High lifetime numbers of sex partners reflect risky sexual behavior and are associated with high HPV infection rates (22). We evaluated associations between the number of lifetime sex partners and persistence; again, no association was found. Finally, to evaluate whether the amount of time between study visits affected detection of persistence, we evaluated whether the number of days between study visits was associated with detection of persistence; no association was found.

TABLE 4. Association between concurrent infection and persistence of high-risk types of human papillomavirus (HPV) among analyzed visit pairs ($n = 181$) contributed by female adolescent study participants, Atlanta, Georgia, 1999–2003

Concurrent infection detected	Univariate OR*		Multivariate OR	
	OR	95% CI*	Adjusted OR	95% CI
Chlamydia trachomatis	1.9	1.0, 3.6	2.1	1.0, 4.1
Neisseria gonorrhoeae	1.4	0.6, 3.3		
Trichomonas vaginalis	1.5	0.5, 5.0		
Bacterial vaginosis	1.3	0.6, 2.7		
More than one type of HPV	2.6	1.5, 4.9	2.8	1.6, 4.9

* OR, odds ratio; CI, confidence interval.

Age, smoking (ever smoking; reported at baseline), oral contraceptive use (90 days prior to the second visit of the pair), parity (ever giving birth; reported at baseline), and douching (90 days prior to the second visit of the pair) may affect cervical and vaginal tissue and/or may have been associated with persistence in other studies. We evaluated associations between these variables and HPV persistence. No association was detected with any of these variables in univariate or multivariate analysis, except smoking. Although smoking was significantly associated with high-risk HPV persistence in univariate analysis, when it was entered into multivariate analysis including concurrent infection with *C. trachomatis* and another HPV type, this variable was not significant, and odds ratios for *C. trachomatis* and more than one HPV type were not altered.

To validate our conclusions, we reran the regression analyses with unstructured and autoregressive correlation structures. The same factors were found to be significant and nonsignificant using these correlation structures.

DISCUSSION

Several studies have demonstrated an association between infection with *C. trachomatis* and cervical cancer independent of HPV status (1, 24, 25); associations between other sexually transmitted infections and cervical cancer have also been reported (2–4, 26–28). We investigated associations between concurrent infection with other sexually transmitted agents and HPV persistence among adolescent girls infected with HPV. In this population, high-risk HPV infections evaluated over 6 months persisted in 43 percent of visit pairs. Persistence was associated with concurrent infection with *C. trachomatis* and concurrent infection with other types of HPV but was not associated with concurrent infection with *T. vaginalis*, *N. gonorrhoea*, or bacterial vaginosis.

The finding that concurrent infection with *C. trachomatis* is associated with HPV persistence accords with studies demonstrating an association between serologic evidence of exposure to *C. trachomatis* and cervical cancer (1, 7, 24). Our findings suggest a mechanism by which concurrent *C. trachomatis* infection may be associated with the development of cervical cancer, since persistence of HPV is the strongest risk factor for the development of cervical cancer (23, 24, 29).

Analyses of the association between *C. trachomatis* and cervical cancer have evaluated women many years after initiation of sexual activity. In this paper, we report an association between *C. trachomatis* and HPV persistence in a relatively young population with a median of 2.6 years since first sex (range, 0.1–5.7 years), which suggests that long-term infection with *C. trachomatis* is not necessary to detect an association between *C. trachomatis* and HPV persistence. Thus, *C. trachomatis* may influence the natural history of HPV infection soon after infection by affecting persistence. In a population in which *C. trachomatis* was associated with cervical cancer, *C. trachomatis* DNA was detected in Papanicolaou smears performed many years before cancer diagnosis but not in Papanicolaou smears performed close in time to the cancer diagnosis (25, 30); this supports the idea that concurrent infection with *C. trachomatis* may affect HPV early in the course of HPV infection.

Other studies have evaluated factors associated with HPV persistence. The only other sexually transmitted agent associated with HPV persistence is human immunodeficiency virus (31, 32). While human immunodeficiency virus infection may have local as well as systemic effects on HPV persistence, the effect of *C. trachomatis* infection is likely to be localized at the cervix.

The association between *C. trachomatis* infection and persistence may reflect host or infectious agent-derived factors. Detection of an association between high-risk HPV persistence and concurrent *C. trachomatis* infection but not *N. gonorrhoea* or *T. vaginalis* infection suggests that the association is due to factors specific to *C. trachomatis* infection; however, the small number of concurrent infections with *N. gonorrhoea* and *T. vaginalis* may have limited our ability to detect an association with HPV persistence.

C. trachomatis infection may increase susceptibility to HPV on a cellular level by increasing access to the basal epithelium due to microabrasions or by altering characteristics of epithelial cells, increasing the viral load of the infection and facilitating persistence. Alternatively, concurrent infection with *C. trachomatis* may impede clearance of HPV by inducing a shift in the immune response to the HPV infection. Cellular (T-helper cell type 1) immune responses have been shown to be important in the clearance of HPV lesions (33). Unresolved *C. trachomatis* infection has been associated with a humoral (T-helper cell type 2) immune response (34, 35), and inflammatory infiltrates seen in *C. trachomatis* infection are characterized by a high proportion of plasma cells, in contrast to the histopathology of gonorrhea infection, wherein a lower proportion of the inflammatory infiltrate is composed of plasma cells (36). Therefore, modulation of the cervical immune response toward a T-helper cell type 2 response may be a *C. trachomatis*-specific effect which increases persistence of HPV. Evaluations of the immunologic milieu surrounding the cervices of coinfected women as compared with women infected with HPV only would test this hypothesis. It is possible that concurrent infection with other sexually transmitted agents may exacerbate the effect of *C. trachomatis* on HPV persistence; further evaluation of concurrent infection with more than one sexually transmitted agent would also be valuable.

The effect of infection with multiple HPV types on persistence may be due to an increased viral load, which has been associated with persistence (8, 37), or it may reflect host susceptibility to both infection and persistence. The importance of host factors in susceptibility to infection with multiple HPV types is underscored by the observation that infection with multiple HPV types occurred more commonly than would be expected if independence of acquisition of the different types was assumed, in an analysis controlling for sexual behavior (38). However, this study did not control for sex partner's age, which was a predictor of baseline HPV infection in our study population (11). Two studies have reported no association between persistence and infection with multiple HPV types (39, 40), while

one other study has reported this association (17). Further evaluation of this issue is needed, particularly because a vaccine for HPV may alter the population of infecting types for vaccinated populations and thereby alter the frequency of infection with multiple types.

Our study had several limitations. We do not know whether these results are generalizable to a population with lower rates of *C. trachomatis* and HPV infection. The relatively short follow-up time limited our ability to evaluate persistence of longer than 6 months. HPV persistence has been evaluated at 4, 6, 9, 12, and 24 months and as being present at two sequential or nonsequential visits, irrespective of the intervening time. Several studies have evaluated 6-month persistence (8, 10, 17, 39), making this measurement useful for comparing results. Cervical cancer develops over a longer period of time, making evaluation of long-term persistence important to confirm the role of *C. trachomatis* in progression to cancer.

Many of the HPV infections we evaluated were initially detected at the baseline visit, making any analysis of incidence impossible and limiting our ability to draw conclusions about duration of infection. In the situation of infection with more than one type of HPV, it is possible that the times of infection differed and one or more of the infections was recently incident. Therefore, persistence of more than 6 months may reflect differing lengths of persistence. Detection of one HPV type at two sequential visits cannot distinguish between persistent infection and reinfection—a limitation shared by all studies of this type. However, lack of a detectable association between the number of sex partners or sex acts and persistence suggests that we detected persistence rather than reinfection. Relatively low numbers of study participants with persistent low-risk HPV infection were expected; this made evaluation of associations with persistence of low-risk types difficult, and the fact that no associations were seen may have been due to sample size as well as biologic differences between high- and low-risk types. The use of more than one *C. trachomatis* or *N. gonorrhoea* test in a subset of our study samples could have created a bias toward detecting these infections at visits with multiple tests; however, because the number of discrepant results between tests was very low, it is likely that any bias introduced was minimal. The test we used to detect *T. vaginalis*, the wet mount technique, is relatively insensitive, and this may have resulted in an inability to detect an association between *T. vaginalis* infection and HPV persistence. Finally, the limited age range in our study made assessment of the effect of age difficult, and we were not able to assess the role of nutritional factors or another coinfection associated with cervical cancer, herpes simplex virus type 2.

Our results demonstrate an association between infection with *C. trachomatis* and outcomes of HPV infection in adolescents with risky sexual behaviors. *C. trachomatis* screening is recommended for all sexually active adolescents. *C. trachomatis* screening coverage among adolescents is estimated to be as low as 27 percent in some states, and the median estimated *C. trachomatis* screening coverage for all states is 60 percent (41). *C. trachomatis* infection is a common sexually transmitted disease with outcomes that include infertility, and concurrent *C. trachomatis* infection may influence HPV outcomes; therefore, efforts need to be made to improve *C. trachomatis* screening coverage.

These results suggest that concurrent infections can affect the host's ability to resolve HPV infection, and as such they emphasize the value of screening and treatment for sexually transmitted infections. This study further demonstrates an effect of concurrent infection with HPV and *C. trachomatis*, supporting a possible role for *C. trachomatis* infection in the development of cervical cancer.

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