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Quantitative Culture of Endocervical *Chlamydia trachomatis*

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We examined the number of *Chlamydia trachomatis* inclusions produced in the initial passage of cell cultures of endocervical specimens from 1,231 women with positive chlamydial cultures who attended a sexually transmitted diseases clinic. Youth, white race, oral contraceptive use, and concurrent infection by *Neisseria gonorrhoeae* were associated with high chlamydial inclusion counts. Youth, white race, and oral contraceptive use were independent determinants of a high chlamydial inclusion count in women without concurrent gonorrhea but not in women with gonorrhea. Results of our study suggest that the degree of chlamydial excretion from the infected cervix may be influenced by characteristics of the patient being tested and may affect the ability to detect *C. trachomatis* in different patient groups.

Infection by *Chlamydia trachomatis* is the most common curable sexually transmitted disease (STD) in the United States (7, 48), with an estimated 4 million cases occurring in adults each year (7). Endocervical infection in women can lead to acute salpingitis, scarring, and dysfunction of the fallopian tubes, with subsequent infertility or ectopic pregnancy (21, 31). The total costs of chlamydial infections in the United States exceed $1 billion annually (50).

In most epidemiologic studies, genital infection by *C. trachomatis* is determined by isolation of the agent in cell culture. Although cell culture is currently the most sensitive method for detecting chlamydial infection, it may be only 70 to 80% sensitive when a single endocervical specimen is tested (10, 11, 28, 39). Because of the expense and limited availability of chlamydial cell culture, its use is often guided by the perceived risk of infection in a given patient. This allows selective diagnostic testing in certain clinical settings, thereby conserving resources.

Studies of risk factors for chlamydial infection in women at high risk for STDs have usually noted the association of endocervical infection with youth, concurrent gonorrhea, and oral contraceptive use (2, 13, 15–18, 27, 32, 38, 41, 42, 45, 46). It is unknown whether the factors associated with higher isolation rates of *C. trachomatis* are a result of increased exposure to the infection, to increased susceptibility to infection once a person is exposed, or to increased detectability of infection because of enhanced chlamydial growth and recovery in culture. We examined the relationships between various epidemiologic variables and the number of organisms detected on first passage in cell culture of specimens obtained from *C. trachomatis*-infected women.

(This study was presented in part at the Seventh Meeting of the International Society for STD Research, Atlanta, Ga., 2 to 5 August 1987.)

MATERIALS AND METHODS

Study population. Women who attended the Marion County Bellflower STD Clinic in Indianapolis, Ind., between 1 September 1983 and 31 October 1985 and who had endocervical specimens obtained for *C. trachomatis* culture were included in the study. Women considered at high risk for infection (7) were cultured for *C. trachomatis* by standard clinic protocols for the collection of specimens. Women considered at high risk were women with known gonorrhea, women who were contacts of men with urethritis, or women with mucopurulent cervicitis on examination (34).

Only the initial visit by each woman who visited the clinic during the study period was used for determining the proportion of women tested for chlamydia. For the purpose of examining the risk of chlamydial infection, a woman was eligible only if a specimen for chlamydial culture was obtained at her first clinic visit during the study period. For the purpose of examining the relationship between the epidemiologic variables and the number of organisms detected in cell culture, only the first positive culture result during the study period was used for any woman. Women who were pregnant or who used an intrauterine contraceptive device were excluded from the study population.

Examination and clinical definitions. The patients were interviewed by using a standard history form. Women were specifically questioned about the reason for their clinic visit, their symptoms, their menstrual and contraceptive histories, and their history of prior STDs.

Clinicians performed a standard, directed genitourinary physical examination of each patient. Clinicians used a cotton-tipped applicator to obtain a specimen from the endocervical canal for *Neisseria gonorrhoeae* culture. After removing cervical mucus and visually examining the woman for signs of cervical or vaginal abnormalities, clinicians used a cotton-tipped wire shaft swab (Medical Wire, Bath, Kent, United Kingdom) to collect a specimen from the endocervical canal for *C. trachomatis* culture. Samples of vaginal secretions were obtained from the posterior vaginal fornix for wet mount examination. Sampling techniques and laboratory procedures were constant throughout the study period.

We defined vaginal or cervical symptoms to be a vaginal discharge, itching, pain, dysuria, dyspareunia, a genital lesion, or a rash. The time since the onset of the patient's last menstrual period was classified into menstrual quintiles (1, 2,

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3, 4, and >4 weeks). Cervical ectopy was considered to be any effacement of the squamocolumnar junction beyond the cervical os (25). The degree of cervical ectopy was defined as the ratio of ectropion to total ectocervical mucosa and was categorized as none, less than one-third, one-third to one-half, and one-half or more. Cervical mucopus was defined as the presence of a yellow or greenish exudate on the first endocervical swab (5). Cervical friability was recorded if cervical bleeding occurred with manipulation during the examination.

Laboratory testing. Wet mount preparations were examined for the presence of motile *Trichomonas vaginalis*. *N. gonorrhoeae* and *C. trachomatis* were isolated and identified as described previously (24). Specimens for culture of *C. trachomatis* were collected into 0.2 M sucrose-phosphate transport medium and stored at 4°C for less than 24 h. Specimens were vortexed, and a 0.1-ml portion was applied to McCoy cell monolayers in each of two wells of a 96-well microdilution plate by the method of Yoder et al. (51). After incubation for 72 h at 35°C, chlamydial inclusions were counted following immunofluorescence staining by using a *Chlamydia* genus-specific monoclonal antibody as described previously (24). The number of inclusions was determined either by counting all inclusions in each well or, for specimens with >10 inclusions per field, by averaging the number of inclusions in each of three fields and multiplying that value by the number of fields per well. The average number of inclusions per well was multiplied by 10 to give the number of inclusion-forming units (IFU) per milliliter of transport medium.

Statistical analysis. We analyzed the number of chlamydial inclusions detected in cultures from infected women as a function of the following independent variables: age; ethnicity; use of oral contraceptives; concurrent infection by *N. gonorrhoeae* or *T. vaginalis*; history of any prior STD; menstrual quintile; genital symptoms; and the presence of signs of cervical ectopy, cervical mucopus, or cervical friability.

Univariate analysis. We used the natural logarithm of the inclusion count (ln IFU per milliliter) as the dependent variable in univariate analysis by each independent variable. Only those inclusion counts determined during the initial passage in cell culture were used. Dichotomous independent variables were analyzed by using Student's *t* test. Discrete variables having more than two outcome categories were examined by analysis of variance.

Univariate analyses of chlamydial isolation rates were performed by chi-square tests. Relative risks and 95% confidence limits (14) for isolation rates were also calculated for each dichotomous factor.

Multivariate analysis. We used stepwise linear regression to determine the best set of independent predictors of inclusion counts. Only predictors that were found to be significant in the univariate analyses were included in the multivariate model. We confirmed each model by using a backward stepwise procedure and examined all two-way interactions among the predictors that were included in the final models.

RESULTS

A total of 9,708 women attended the clinic for an initial visit during the study period. Chlamydial culture results were available for 6,315 women (65.0%) tested at any time during the study period. Of these, 5,276 eligible women (54.3%) were tested on their first visits during the study period. Clinicians followed criteria for selective laboratory screening for *C. trachomatis* (7): younger women, oral contraceptive users, and women without genital symptoms were more likely to be tested than were other women (Table 1). In addition, women with signs of mucopurulent cervicitis (5) and women who were eventually found to have concurrent gonorrhea were more likely to be cultured for *C. trachomatis*, reflecting the definition of high risk used to select women for culture.

Endocervical *C. trachomatis* was detected in the initial passage of cell culture in 1,300 of 5,276 (24.6%) of the study population. Of the infected women, 780 were black, 516 were white, and 4 had no racial data recorded (see Table 3). Of the chlamydia-infected women, 599 (46%) were also infected with *N. gonorrhoeae*. Oral contraceptive use was reported by 1,769 (33.5%) of the subjects. Exclusion of pregnant women and women who used intrauterine contra-

TABLE 1. Proportion of women tested for *C. trachomatis* by epidemiologic characteristics, Bellflower STD Clinic, Indianapolis, 1983 to 1985

Variable	No. of women cultured/no. of women seen (%)
Age (yr)	
<20	1,233/2,074 (59.4)
20–25	2,349/4,052 (58.0)
≥26	1,694/3,581 (47.3)
Oral contraceptive use	
User	1,769/3,036 (58.3)
Nonuser	3,507/6,671 (52.6)
Ethnicity	
Black	3,197/5,838 (54.8)
White	2,071/3,861 (53.6)
Concurrent infection	
Gonorrhea	1,965/3,058 (64.3)
No gonorrhea	3,311/6,649 (49.8)
Trichomoniasis	923/1,639 (56.3)
No trichomoniasis	3,864/6,471 (59.7)
STD history	
Prior STD	1,774/3,491 (50.8)
No prior STD	3,501/6,215 (56.3)
Vaginal symptoms	
Present	2,459/5,110 (48.1)
Absent	2,816/4,592 (61.3)
Cervical signs	
Ectopy	
Present	425/558 (76.2)
Absent	4,704/7,756 (60.6)
Mucopus	
Present	686/1,009 (68.0)
Absent	3,757/6,231 (60.3)
Friability	
Present	1,380/2,671 (51.7)
Absent	3,623/5,424 (66.8)

TABLE 2. Univariate analysis of factors associated with numbers of chlamydial inclusions in endocervical specimens from women attending Bellflower STD Clinic, Indianapolis, 1983 to 1985

Variable	No. of women	Geometric mean IFU/ml	P
Age (yr)			
<20	453	742	
20–25	565	528	0.006
≥26	212	433	
Oral contraceptive use			
User	552	773	<0.001
Nonuser	678	455	
Ethnicity			
Black	791	508	0.005
White	438	735	
Concurrent infection			
Gonorrhea	564	706	0.003
No gonorrhea	666	488	
Trichomoniasis	213	384	0.002
No trichomoniasis	893	646	
STD history			
Prior STD	474	473	0.014
No prior STD	756	652	
Vaginal symptoms			
Present	518	596	0.68
Absent	712	567	
Cervical signs			
Ectopy			
Present	146	713	0.23
Absent	1,055	567	
Mucopus			
Present	185	550	0.92
Absent	864	539	
Friability			
Present	381	608	0.83
Absent	782	590	

ceptive devices yielded 1,231 chlamydia-infected women for analysis of inclusion count data.

The proportions of women with various inclusion counts were as follows: ≤100 IFU/ml, 25.0%; 101 to 1,000 IFU/ml, 39.8%; 1,001 to 10,000 IFU/ml, 21.1%; >10,000 IFU/ml, 14.1%.

Univariate analysis of inclusion counts. Univariate analysis revealed that specimens from younger women yielded significantly higher inclusion counts than did those from older infected women (Table 2). The geometric mean inclusion count decreased with age from 742 for women less than 20 years old to 433 for women greater than 26 years old. Higher inclusion counts were also noted in specimens from women who used oral contraceptives (geometric mean, 773) than in women who did not use oral contraceptives (geometric mean, 455). Higher inclusion counts were also associated with concurrent gonorrhea and white ethnicity.

Chlamydial inclusion counts were lower in women with a history of STDs than in women with no history of STDs. Infected black women were more likely than infected white women to have a history of STDs (348 of 791 [44%] black women versus 126 of 438 [29%] white women; $P < 0.001$). The presence of vaginal trichomoniasis was also associated with lower chlamydial inclusion counts. We noted no difference in inclusion counts by vaginal or cervical symptoms or cervical signs (Table 2). In addition, there was no difference in inclusion counts by menstrual cycle quintile (data not shown).

Among the chlamydia-infected women, the racial proportions, age characteristics, and patterns of oral contraceptive use were similar in gonorrhea-positive and gonorrhea-negative women. Trichomoniasis occurred more frequently in women with gonorrhea than in women without gonorrhea. Of 494 women with gonorrhea and a valid wet mount result, 115 (23.3%) were also infected with *T. vaginalis*, compared with 97 of 600 women (16.2%) without gonorrhea ($P = 0.004$). When analyzed as a dichotomous variable (less than one-half or greater than or equal to one-half effacement), cervical ectopy was associated with increased chlamydial inclusion counts in women without gonorrhea ($P = 0.034$). This association was not significant in women with concurrent gonorrhea. When cervical ectopy was analyzed as a ranked categorical variable (no effacement or less than or equal to one-third, one-third to one-half, or greater than or equal to one-half effacement), a monotonic increase in inclusion counts was seen among women without gonorrhea, although the differences did not achieve statistical significance ($P = 0.077$). Neither mucopus nor cervical friability was associated with higher inclusion counts.

Multivariate analysis of inclusion counts. Variables with significant univariate associations with inclusion count (ethnicity, age, oral contraceptive use, concurrent gonorrhea or trichomoniasis, and history of prior STDs) and the presence of cervical ectopy were evaluated in our regression model. Age, ethnicity, oral contraceptive use, concurrent gonorrhea, and concurrent trichomoniasis were independently associated with the inclusion count in the multivariate analysis. No two-way interactions significantly improved the predictive value of the model. When the study population was divided according to the presence of concurrent gonorrhea, age, ethnicity, and oral contraceptive use remained significantly associated with the inclusion count in women without concurrent gonorrhea. In women with gonorrhea, only concurrent trichomoniasis was associated with the inclusion count.

Risk factors for chlamydial infection. Risk factors for the isolation of endocervical *C. trachomatis* were youth; oral contraceptive use; concurrent gonorrhea; and the presence of cervical signs of ectopy, mucopus, or friability (Table 3). The relative risks for each of these variables were, however, small, indicating the homogeneous, high-risk nature of the portion of the clinic population selected for testing.

DISCUSSION

We found several variables to be associated with differences in the recovery of endocervical chlamydia in cell culture as measured by inclusion count. There is great variability in inclusion counts obtained from replicate cell cultures of identical clinical specimens (22). We therefore used a large set of chlamydia-infected women to test for

TABLE 3. Relative risks for endocervical *C. trachomatis* infection among women attendees at Bellflower STD Clinic, Indianapolis, 1983 to 1985

Variable	No. with positive *C. trachomatis* culture/no. cultured (%)	RR (CI95)[a]
Age (yr)		
<20	454/1,233 (36.8)	2.9 (2.5, 3.3)
20–25	599/2,349 (25.5)	1.8 (1.5, 2.0)
≥26	247/1,694 (14.5)	1.0 (reference)
Oral contraceptive use		
User	562/1,769 (31.8)	1.5 (1.4, 1.7)
Nonuser	738/3,507 (21.0)	
Ethnicity		
White	516/2,071 (24.9)	1.0 (0.9, 1.1)
Black	780/3,197 (24.3)	
Concurrent infection		
Gonorrhea	599/1,965 (30.5)	1.4 (1.3, 1.6)
No gonorrhea	701/3,311 (21.2)	
Trichomoniasis	241/923 (26.1)	1.1 (1.0, 1.2)
No trichomoniasis	931/3,864 (24.1)	
STD history		
Prior STD	356/1,774 (20.1)	0.8 (0.7, 0.8)
No prior STD	943/3,501 (26.9)	
Vaginal symptoms		
Present	566/2,459 (23.0)	0.9 (0.8, 1.0)
Absent	734/2,816 (26.1)	
Cervical signs		
Ectopy		
Present	169/425 (39.7)	1.7 (1.5, 1.9)
Absent	1,095/4,704 (23.2)	
Mucopus		
Present	199/686 (29.0)	1.2 (1.1, 1.4)
Absent	904/3,757 (24.1)	
Friability		
Present	403/1,380 (29.2)	1.3 (1.2, 1.4)
Absent	824/3,623 (22.7)	

[a] RR (CI95), Relative risk (95% confidence interval). Women ≥26 years old were assumed to have a relative risk of 1.00.

associations with a higher statistical power than those in prior studies.

The results of our univariate analysis showed higher numbers of chlamydial inclusions from infected women who were younger, used oral contraceptives, were white, or who had concurrent infection by *N. gonorrhoeae*. A history of prior STDs or the presence of concurrent infection by *T. vaginalis* were associated with lower inclusion counts. Biologically plausible hypotheses can be suggested to explain each of these associations.

Age. Several studies have found age to be inversely correlated with the prevalence of endocervical *C. trachomatis* infection (15–18, 38, 41, 45). Sexually active women under the age of 20 have a two- to threefold higher risk of infection than do older women (49), although some studies of adolescents have not found this association (8, 42). A similar relationship of age and infection has been seen in males (37). Acquired immunity to chlamydia, with increasing age reflecting increased exposure and subsequent resistance to chlamydia, may explain these observations. Our study shows that not only are chlamydial isolation rates lower among older women, but the number of chlamydia recovered from infected older women is less than that recovered from younger women. Partial immunity acquired through previous chlamydial infection may decrease the excretion of endocervical *C. trachomatis*. This could serve to obscure infection in some women whose chlamydial excretion is below the sensitivity of cell culture. Developmental rather than immunologic phenomena could also explain our findings. Changes in the cervical epithelium related to age and not reflected in our gross clinical measurement of cervical ectopy might cause increased chlamydial replication, maturation, and excretion in younger women.

Oral contraceptive use. Results of our study confirm the results of a smaller previous study (30) in that we found higher chlamydial inclusion counts in women who used oral contraceptives. Higher chlamydial isolation rates have also been consistently noted among women who use oral contraceptives (2, 13, 15–18, 20, 27, 30, 32, 37, 38, 41, 42, 45, 46, 48, 49). Oral contraceptives may predispose a woman to the acquisition of infection, increase the excretion of chlamydia at the endocervix and the sensitivity of the chlamydia culture system, or both (41, 49). Laboratory studies have provided support for both explanations. Treatment with estrogen facilitates the development of *C. trachomatis* in vitro and *Chlamydia psittaci* in animal models of chlamydial infection (6, 35). Cervical ectopy is more frequent in adolescent women and oral contraceptive users (25). Thus, the effect of oral contraceptive use and age on chlamydial isolation may operate through promoting cervical ectopy. This could simply mechanically increase the area of cervical epithelium exposed for infection or sampling. Alternatively, there may be a biologic enhancement of *C. trachomatis* excretion (49). We found no association between cervical ectopy and increased chlamydial inclusion counts, although both youth and oral contraceptive use were associated with higher counts. These findings suggest that age and oral contraceptive use may directly influence chlamydial excretion rather than being covariates for the presence of cervical ectopy.

We found no differences in chlamydial inclusion counts by menstrual cycle interval among women who did not use oral contraceptives. This suggests that physiologic hormonal fluctuations may have little influence on the isolation of endocervical chlamydiae.

Ethnicity. White women with endocervical *C. trachomatis* infection had higher chlamydial inclusion counts than did infected black women, although the magnitude of the difference was less than that observed for the extreme age strata and for oral contraceptive use. In contrast to previous studies which found higher isolation rates in black women (32, 33), we observed no difference in the isolation rates of endocervical *C. trachomatis* between white women and black women. We believe that differences in isolation rates by race are more likely mediated by exposure variables and by practices of seeking health care than by biologic differences in organism excretion. It is possible that the duration of infection, which is impossible to determine because of the asymptomatic nature of most *C. trachomatis* endocervical infections, may determine the level of excretion and, hence, the detectability of infection. If so, the practices of seeking

health care might be confounders in infection status. Prior studies in Indianapolis have observed important influences of race, educational level, and socioeconomic status on the speed with which patients seek care for STDs (3). Alternatively, the ethnic differences in inclusion counts and isolation rates could be due to an influence of *C. trachomatis* serotype on excretion and recovery of the organism in cell culture. If this occurs and the chlamydial serotype distribution is geographically and ethnically determined, apparent differences in both inclusion counts and isolation rates could occur among studies conducted in different populations.

Prior STD. Chlamydial isolation rates are lower in patients with a history of STDs (26), and this finding was observed in the study population described here. In addition, our univariate analysis showed lower chlamydial inclusion counts in women reporting prior STDs. The lack of information regarding specific STDs and the relationship of self-reported STDs to chlamydial isolation and inclusion counts requires more detailed study.

Concurrent STD. Results of our study confirm those of several other studies (18, 30) in that we found higher chlamydial isolation rates in women with concurrent gonorrhea. In addition to the epidemiologic association because of concurrent exposure between these two sexually acquired infections, it is possible that infection by *N. gonorrhoeae* may reactivate latent chlamydial infection. We observed an association between concurrent gonorrhea and increased chlamydial inclusion counts that was not noted previously (20). The presence of higher chlamydial inclusion counts in women with gonorrhea suggests that chlamydial shedding may be increased by concurrent *N. gonorrhoeae* infection. Richmond et al. (37) noted an increased frequency of isolation of *C. trachomatis* in men with concurrent *N. gonorrhoeae* infection. However, *C. trachomatis* inclusion counts are lower in urethral cultures from men with concurrent infection by *N. gonorrhoeae* than they are in urethral cultures from men without gonorrhea (30, 43). The mechanism whereby *N. gonorrhoeae* might increase the excretion of endocervical *C. trachomatis* is unclear. Recovery of *C. trachomatis* from the endocervix is inversely proportional to the presence of local antibody, which could be reduced by the proteases produced by *N. gonorrhoeae* (4). Further studies are needed to define a biologic relationship between chlamydial excretion and infection by *N. gonorrhoeae*.

Although concurrent gonorrhea was associated with higher chlamydial inclusion counts in the univariate analysis, the relationships of age, oral contraceptive use, and ethnicity to chlamydial recovery were not noted in the women with concurrent gonorrhea. Concurrent infection by *N. gonorrhoeae* may increase chlamydial excretion from the chlamydia-infected cervix to such an extent that differences in inclusion counts as a result of other influences are not detected. In contrast, concurrent trichomoniasis, which was associated with low chlamydial inclusion counts in the univariate analysis, remained associated with chlamydial inclusion counts in multivariate analysis of women with concurrent gonorrhea. This suggests that concurrent trichomoniasis is not simply a covariate of age, oral contraceptive use, or ethnicity and may exert an influence on chlamydial inclusion counts through a different mechanism than these variables. Our finding that vaginal trichomoniasis was associated with reduced chlamydial inclusion counts confirms the findings of an earlier study (30). *T. vaginalis* is known to cause a leukocytic response in patients with vulvovaginitis, and the inflammatory cells might decrease chlamydial infectivity in vitro. In addition, *T. vaginalis* is cytotoxic to cell monolayers, such as those used for the isolation of *C. trachomatis* (1). This could result in a lower number of cells that support chlamydial inclusions in the cell culture system. A direct effect of *T. vaginalis* on chlamydia-infected cells is less likely, because the columnar epithelium of the endocervix is resistant to infection by *T. vaginalis* (36).

Isolation rates of *C. trachomatis*. Various epidemiologic characteristics have been associated with increased isolation rates of *C. trachomatis* in other studies (2, 13, 15–18, 27, 29, 32, 38, 41, 42, 45, 46, 48). Isolation rates are complex outcomes which are influenced by many factors. Risk of exposure reflects behavioral influences such as sexual activity and choice of sexual partner. Risk of infection following exposure reflects biologic susceptibility and resistance of the host to infection. Finally, detection of infection reflects the sensitivity of the clinical or laboratory test for infection. We studied a group of STD clinic attendees who were tested for *C. trachomatis* cervical infection. Within this tested group, we examined isolation rates and compared the factors for increased risk of infection with those factors found among infected women to influence the recovery of *C. trachomatis*, as measured by inclusion counts. The factors which we found to influence isolation rates (chance of infection) were similar, with the exception of ethnicity, to factors associated with increased isolation rates in prior studies. In our study, the similarity in factors that were associated with infection and that were also associated with increased numbers of chlamydial inclusions in cell cultures suggests that women who are younger, use oral contraceptives, or (in Indianapolis) are white may be detected more easily by the methods used in our study. The quantitative effect of each of the factors associated with chlamydial inclusion counts requires further study. It is possible that the influences described in this report are greater in chlamydial detection systems in which the initial sensitivity is low. The chlamydial culture system used during this study was less sensitive than other, more laborious methods of cell culture (23, 40). However, we believe our findings may be applicable to other clinical laboratory methods of chlamydial detection because our findings are similar to those of prior studies in which different culture systems were used (20, 30). In addition, the sensitivity of all methods of cell culture on which current risk factors for chlamydial infection are derived is also limited (9–11, 19, 28, 39). Other laboratories that have used different comparative detection methods (28, 47) have found sensitivities of chlamydial antigen detection tests that were similar to those found in our laboratory (44), suggesting the equivalence of the cell culture methods. Magder et al. (29), using a different cell culture method, did not demonstrate associations of patient attributes with chlamydial inclusion counts; however, data from only 172 culture-positive women were analyzed. The study of Magder et al. (29) also suggested the limited sensitivity of a single endocervical swab for detecting *C. trachomatis*: 40% of positive isolates exhibited $\leq$10 inclusions; moreover, a 13% increase in case findings was realized if a second endocervical swab was obtained and a 19% increase was found if a urethral swab was obtained from women.

The magnitude of the effect of patient characteristics on the detectability of chlamydial infection is not known but varies with the true sensitivity of the detection system that is used. The primary goal of our study was to examine the differential detectability of *C. trachomatis* because of factors that influence cell culture isolation. Our findings suggest that age, oral contraceptive use, ethnicity, and the presence of concurrent STDs may influence the recovery of chlamydia in

cell cultures. Any biologic reasons for these associations between patient attributes and chlamydial inclusion counts remain unknown. The chlamydial antigen detection systems that are becoming popular are less sensitive than cell culture and may be more likely to show the effect of differential detectability in younger women and oral contraceptive users (44; J. S. Lin, E. E. Gray, R. M. Haivanis, W. E. Jones, and P. A. Rice, Seventh Meeting of the International Society for STD Research, Atlanta, Ga., 2 to 5 August 1987). Further studies are necessary to determine the magnitude of this bias on relative risk estimates for chlamydial infection (12). However, our study suggests that epidemiologic studies must be interpreted cautiously when the classification of patient diagnosis is based on an insensitive measure of disease.

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LITERATURE CITED

1. **Alderete, J. F., and E. Pearlman.** 1983. Pathogenic *Trichomonas vaginalis* cytotoxicity to cell culture monolayers. Br. J. Vener. Dis. **60**:99–105.
2. **Arya, O. P., H. Mallinson, and A. D. Goddard.** 1981. Epidemiological and clinical correlates of chlamydia infection of the cervix. Br. J. Vener. Dis. **47**:118–124.
3. **Brooks, G. F., W. W. Darrow, and J. A. Day.** 1978. Repeated gonorrhea: an analysis of importance and risk factors. J. Infect. Dis. **137**:161–169.
4. **Brunham, R. C., C. C. Kuo, L. Cles, and K. K. Holmes.** 1983. Correlation of host immune response with quantitative recovery of *Chlamydia trachomatis* from the human endocervix. Infect. Immun. **39**:1491–1494.
5. **Brunham, R. C., J. Paavonen, C. E. Stevens, N. Kiviat, C. C. Kuo, C. W. Critchlow, and K. K. Holmes.** 1984. Mucopurulent cervicitis—the ignored counterpart in women of urethritis in men. N. Engl. J. Med. **311**:1–6.
6. **Bushell, A. C., and D. Hobson.** 1978. Effect of cortisol on growth of *C. trachomatis* in McCoy cells. Infect. Immun. **21**:946–953.
7. **Centers for Disease Control.** 1985. *Chlamydia trachomatis* infections. Policy guidelines for prevention and control. Morbid. Mortal. Weekly Rep. **34**(Suppl. 3):53S–74S.
8. **Chacko, M. R., and J. C. Lovchik.** 1984. *Chlamydia trachomatis* infection in sexually active adolescents: prevalence and risk factors. Pediatrics **73**:836–840.
9. **Chernesky, M. A., J. B. Mahony, S. Castriciano, M. Mores, I. O. Stewart, S. J. Landis, W. Seidelman, E. J. Sargeant, and C. Leman.** 1986. Detection of *Chlamydia trachomatis* antigens by enzyme immunoassay and immunofluorescence in genital specimens from symptomatic and asymptomatic men and women. J. Infect. Dis. **154**:141–148.
10. **Dunlop, E. M. C., B. T. Goh, S. Darougar, and R. Woodland.** 1985. Triple-culture tests for diagnosis of chlamydial infection of the female genital tract. Sex. Transm. Dis. **12**:68–71.
11. **Embil, J. A., H. J. Thiebauz, F. R. Manuel, L. H. Pereira, and S. W. MacDonald.** 1983. Sequential cervical specimens and the isolation of *Chlamydia trachomatis*: factors affecting detection. Sex. Transm. Dis. **10**:62–66.
12. **Fleiss, J. L.** 1981. Statistical methods for rates and proportions, 2nd ed., p. 188–211. John Wiley & Sons, Inc., New York.
13. **Fraser, J. J., P. J. Rettig, and D. W. Kaplan.** 1983. Prevalence of cervical *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in female adolescents. Pediatrics **71**:333–336.
14. **Greenland, S., and J. M. Robins.** 1985. Estimation of a common effect parameter from sparse followup data. Biometrics **41**: 55–68.
15. **Handsfield, H. H., L. L. Jasman, P. L. Roberts, V. W. Hanson, R. L. Kothenbeutel, and W. E. Stamm.** 1986. Criteria for selective screening for *Chlamydia trachomatis* infection in women attending family planning clinics. J. Am. Med. Assoc. **255**:1730–1734.
16. **Harrison, H. R., E. R. Alexander, L. Weinstein, M. Lewis, M. Nash, and D. A. Sim.** 1983. Cervical *Chlamydia trachomatis* and mycoplasmal infections in pregnancy. J. Am. Med. Assoc. **250**:1721–1727.
17. **Harrison, H. R., M. Costin, J. B. Meder, L. M. Bownds, D. A. Sim, M. Lewis, and E. R. Alexander.** 1985. Cervical *Chlamydia trachomatis* infection in university women: relationship to history, contraception, ectopy, and cervicitis. Am. J. Obstet. Gynecol. **153**:244–251.
18. **Hilton, A. L., S. J. Richmond, J. D. Milne, F. Hindley, and S. K. R. Clarke.** 1974. *Chlamydia* A in the female genital tract. Br. J. Vener. Dis. **50**:1–10.
19. **Hipp, S. S., H. Yangsook, and D. Murphy.** 1987. Assessment of enzyme immunoassay and immunofluorescence test for detection of *Chlamydia trachomatis*. J. Clin. Microbiol. **25**:1938–1943.
20. **Hobson, D., P. Karayiannis, R. E. Byng, E. Rees, A. I. Tait, and J. A. Davies.** 1980. Quantitative aspects of chlamydial infection of the cervix. Br. J. Vener. Dis. **56**:156–162.
21. **Holmes, K. K., D. A. Eschenbach, and J. S. Knapp.** 1980. Salpingitis: overview of etiology and epidemiology. Am. J. Obstet. Gynecol. **138**:893–900.
22. **Johnson, F. W. A., and D. Hobson.** 1976. Factors affecting the sensitivity of replicating McCoy cells in the isolation and growth of chlamydia A (TRIC agents). J. Hyg. **76**:441–451.
23. **Jones, R. B., B. P. Katz, B. Van Der Pol, V. A. Caine, B. E. Batteiger, and W. J. Newhall.** 1986. Effect of blind passage and multiple sampling on recovery of *Chlamydia trachomatis* from urogenital specimens. J. Clin. Microbiol. **24**:1029–1033.
24. **Jones, R. B., R. A. Rabinovitch, B. P. Katz, B. E. Batteiger, T. S. Quinn, P. Terho, and M. A. Lapworth.** 1985. *Chlamydia trachomatis* in the pharynx and rectum of heterosexual patients at risk for genital infection. Ann. Intern. Med. **102**:757–762.
25. **Jordan, J. A., and A. Singer.** 1976. Effect of oral contraceptive steroids upon epithelium and mucus, p. 192–207. *In* J. A. Jordan and A. Singer (ed.), The cervix. The W. B. Saunders Co., London.
26. **Katz, B. P., B. E. Batteiger, and R. B. Jones.** 1987. Effect of prior sexually transmitted disease on the isolation of *Chlamydia trachomatis*. Sex. Transm. Dis. **14**:160–164.
27. **Kinghorn, G. R., and M. A. Waugh.** 1981. Oral contraceptive use and prevalence of infection with *Chlamydia trachomatis* in women. Br. J. Vener. Dis. **57**:187–190.
28. **Lefebvre, J., H. Laperiere, H. Rousseau, and R. Masse.** 1988. Comparison of three techniques for detection of *Chlamydia trachomatis* in endocervical specimens from asymptomatic women. J. Clin. Microbiol. **26**:726–731.
29. **Magder, L. S., H. R. Harrison, J. M. Ehret, T. S. Anderson, and F. N. Judson.** 1988. Factors related to genital *Chlamydia trachomatis* and its diagnosis by culture in a sexually transmitted diseases clinic. Am. J. Epidemiol. **28**:298–308.
30. **Mallinson, H., O. P. Arya, and A. D. Goddard.** 1982. Quantitative study of *Chlamydia trachomatis* in genital infection. Br. J. Vener. Dis. **58**:36–39.
31. **Mårdh, P. A., T. Ripa, L. Svensson, and L. Westrom.** 1977. *Chlamydia trachomatis* infection in patients with acute salpingitis. N. Engl. J. Med. **296**:1377–1379.
32. **McCormack, W. M., B. Rosner, D. E. McComb, J. R. Evrard, and S. H. Zinner.** 1985. Infection with *Chlamydia trachomatis* in female college students. Am. J. Epidemiol. **121**:107–115.
33. **Moscicki, B., M. A. Shafer, S. G. Millstein, C. E. Irwin, and J. Schachter.** 1987. The use and limitations of endocervical Gram stains and mucopurulent cervicitis as predictors for *Chlamydia trachomatis* in female adolescents. Am. J. Obstet. Gynecol. **157**:65–71.
34. **Nettleman, M. D., R. B. Jones, S. D. Roberts, B. P. Katz, A. E. Washington, R. S. Dittus, and T. S. Quinn.** 1986. Cost-effec-

tiveness of culturing for *Chlamydia trachomatis*. A study in a clinic for sexually transmitted diseases. Ann. Intern. Med. **105**:189–196.
35. **Rank, R. G., and A. L. Barron.** 1987. Specific effect of estradiol on the genital mucosal antibody response in chlamydial ocular and genital infections. Infect. Immun. **55**:2317–2319.
36. **Rein, M. F., and M. Muller.** 1984. Trichomonas vaginalis, p. 531. *In* K. K. Holmes, P. A. Mardh, P. F. Sparling, and P. J. Wiesner (ed.), Sexually transmitted diseases. McGraw-Hill Book Co., New York.
37. **Richmond, S. J., A. L. Hilton, and S. K. R. Clarke.** 1972. Chlamydial infection: role of Chlamydia subgroup A in nongonococcal and post-gonococcal urethritis. Br. J. Vener. Dis. **48**:437–444.
38. **Ripa, K. T., L. Svensson, P. A. Mårdh, and L. Westrom.** 1978. *Chlamydia trachomatis* in gynecologic outpatients. Obstet. Gynecol. **52**:698–702.
39. **Schachter, J.** 1984. Biology of *Chlamydia trachomatis*, p. 243–257. *In* K. K. Holmes, P.-A. Mardh, P. F. Sparling, and P. J. Wiesner (ed.), Sexually transmitted diseases. McGraw-Hill Book Co., New York.
40. **Schachter, J., and D. H. Martin.** 1987. Failure of multiple passages to increase chlamydial recovery. J. Clin. Microbiol. **25**:1851–1853.
41. **Schachter, J., E. Stoner, and J. Moncada.** 1983. Screening for chlamydial infections in women attending family planning clinics. West. J. Med. **138**:375–379.
42. **Shafer, M. A., A. Beck, B. Blain, P. Dole, C. E. Irwin, R. Sweet, and J. Schachter.** 1984. *Chlamydia trachomatis*: important relationships to race, contraception, lower genital tract infection, and Papanicolaou smear. J. Pediatr. **104**:141–146.
43. **Singal, S. S., R. C. Reichman, P. S. Graman, C. Greisberger, M. A. Trupei, and M. A. Menegus.** 1986. Isolation of *Chlamydia trachomatis* from men with urethritis: relative value of one vs two swabs and influence of concomitant gonococcal infection. Sex. Transm. Dis. **13**:50–52.
44. **Smith, J. W., R. E. Rogers, B. P. Katz, J. F. Brickler, P. L. Lineback, B. Van Der Pol, and R. B. Jones.** 1987. Diagnosis of chlamydial infection in women attending antenatal and gynecologic clinics. J. Clin. Microbiol. **25**:868–872.
45. **Svensson, L., L. Westrom, and P. A. Mårdh.** 1981. *Chlamydia trachomatis* in women attending a gynaecological outpatient clinic with lower genital tract infection. Br. J. Vener. Dis. **57**:259–262.
46. **Tait, I. A., E. Rees, D. Hobson, R. E. Byng, and M. C. K. Tweedie.** 1980. Chlamydial infection of the cervix in contacts of men with nongonococcal urethritis. Br. J. Vener. Dis. **56**:37–45.
47. **Taylor-Robinson, D., B. J. Thomas, and M. F. Osborn.** 1987. Evaluation of enzyme immunoassay (Chlamydiazyme) for detecting *C. trachomatis* in genital tract specimens. J. Clin. Pathol. **40**:194–199.
48. **Thompson, S. E., and A. E. Washington.** 1983. Epidemiology of sexually transmitted *Chlamydia trachomatis* infections. Epidemiol. Rev. **5**:96–123.
49. **Washington, A. E., S. Gove, J. Schachter, and R. L. Sweet.** 1985. Oral contraceptives, *Chlamydia trachomatis* infection, and pelvic inflammatory disease. J. Am. Med. Assoc. **253**:2246–2250.
50. **Washington, A. E., R. E. Johnson, and L. L. Sanders.** 1987. *Chlamydia trachomatis* infections in the United States: what are they costing us? J. Am. Med. Assoc. **257**:2070–2072.
51. **Yoder, B. L., W. E. Stamm, C. M. Koester, and E. R. Alexander.** 1981. Microtest procedure for isolation of *Chlamydia trachomatis*. J. Clin. Microbiol. **13**:1036–1039.