Comparison of *Chlamydia trachomatis* Serovars Causing Rectal and Cervical Infections

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We used monoclonal antibodies capable of distinguishing serovars of *Chlamydia trachomatis* to compare 314 cervical isolates with 150 rectal isolates from homosexual men. The isolates were obtained from patients attending a sexually transmitted diseases clinic over a two-year study period. The serovar distribution of cervical and rectal isolates differed significantly. Serovar D'/D was found in 53% of the rectal isolates but in only 18% of cervical isolates (*P* < .0001). Serovar E was the predominant serovar in cervical isolates (32%) but was found in only 6% of rectal isolates (*P* < .0001). Serovars B, 1/1', H, and K were isolated from 2%-7% of cervical specimens but were not found in rectal isolates. There was a significant decline in the proportion of rectal infections caused by serovar D'/D over the study period, and clustering of infections caused by other serovars was observed. Serotyping using monoclonal antibodies provides a powerful tool for investigating the epidemiology of sexually transmitted *C. trachomatis* infections.

Although *Chlamydia trachomatis* is the most common sexually transmitted pathogen in the United States [1], few studies have examined patterns of genital transmission because of the lack of readily available bacterial markers to distinguish infecting strains. To date, serotyping by micro-immunofluorescence has been the only system for characterizing strains of *C. trachomatis* in epidemiological studies [2]. Only limited numbers of strains, however, have been characterized by this laborious method [3]. Wang et al. [4] recently described the development of a battery of murine monoclonal antibodies capable of distinguishing *C. trachomatis* serovars. Using these antibodies, we developed a dot-ELISA that makes typing of large numbers of *C. trachomatis* isolates for epidemiological studies feasible [5]. Using this assay, we have studied the distribution of *C. trachomatis* serovars in two populations unlikely to be infected by the same source-contact group: homosexual men and heterosexual women. We hypothesized that because homosexual men are segregated by their sex practices from heterosexual women, comparing organisms from these two populations would be most likely to reveal differences in the occurrence of serovars due to patterns of transmission.

**Subjects and Methods**

**Study population and subject selection.** The Harborview Medical Center–Seattle-King County STD Clinic provides low-cost care for sexually transmitted disease (STD) to ~15,000 patients each year. During the two-year study period (May 1982–April 1984), 25%-30% of visits to the clinic were made by homosexual or bisexual men and 20%-25% by heterosexual women. Patients presenting to the clinic were evaluated by clinicians according to standing clinic orders. Demographic, sexual, and medical histories were taken by a clinical specialist, and a genitourinary examination and collection of specimens for microbiological studies were then done. Endocervical samples for culture of *Neisseria gonorrhoeae* and *C. trachomatis* were collected from all female patients presenting for an initial clinical assessment. Rectal swabs were obtained from women for *C. trachomatis* culture at the discretion of the clinician. Urethral and rectal samples for culture of
N. gonorrhoeae and C. trachomatis were routinely obtained from all homosexual and bisexual men attending the clinic for an initial assessment. All medical records from men with C. trachomatis rectal infection and 118 randomly selected medical records from the women with C. trachomatis cervical infection whose isolates were selected for typing were reviewed to obtain clinical and demographic data on the subjects. Cervical infection was considered symptomatic if the patient's record noted vulvar or vaginal pruritus, vaginal discharge, genital pain, dyspareunia, dysuria, or lower abdominal pain. Rectal infection was considered symptomatic if rectal discharge, bleeding, pain, tenesmus, or pruritus were recorded.

Laboratory records were used to identify all male patients who had C. trachomatis cultured from the rectum during the study period. After identification of a stored rectal isolate, two cervical isolates were selected randomly from stored specimens collected within 30 days of the date of collection of the rectal specimen. In all cases, isolates collected within a year of the collection of another isolate from the same patient were excluded from analysis (five rectal isolates from men and five cervical isolates). No reported isolates were identified as belonging to sex partners who were also within the study population. For comparison, 77 rectal isolates were randomly selected from women with rectal C. trachomatis infections identified during 1984.

Isolation of C. trachomatis. Rectal and cervical specimens for culture of C. trachomatis were obtained with a Type III calcium-alginate swab (Spectrum Laboratories, Los Angeles) and were stored at 4°C in a 0.2 M sucrose/phosphate transport medium containing vancomycin and gentamicin until transported to the laboratory. Specimens were inoculated within 24 hr of collection onto cycloheximide-treated McCoy cell monolayers in 96-well tissue-culture plates as previously described [6]. A 0.1-ml aliquot of the clinical specimen was inoculated, and the remainder of the specimen was frozen at -70°C. All rectal specimens were sonicated before inoculation to reduce contamination by fecal microorganisms and to improve sensitivity [7]. Chlamydial inclusions were detected by direct immunofluorescence after 48–72 hr of intracellular development as previously described [8].

Serovar determination. Cultures identified through laboratory records as positive for C. trachomatis were thawed and passaged in McCoy cell monolayers in 12-mm shell vials until highly infectious (~10⁷ infectious units). Of 204 available rectal isolates from homosexual or bisexual men, 159 (78%) were successfully recovered after freezer storage, and antigen sufficient in quantity for serotyping was obtained from 155 of these isolates. Recovery of cervical isolates was more successful; of 335 identified cultures, 328 (98%) were viable, and 319 produced a sufficient amount of antigen for typing. Five rectal and five cervical isolates were from patients with other isolates already included in the study; these were excluded from further consideration. Thus, 150 rectal and 314 cervical isolates provided the final material for typing. Of the 77 rectal isolates from women, only 32 (42%) were successfully recovered for typing.

Chlamydial antigen for serotyping was obtained by sonicating vial monolayers then centrifuging them. Isolates were serotyped by a dot-ELISA using monoclonal antibodies specific for type- and subspecies-specific epitopes of C. trachomatis as previously described [5].

Statistical methods. The statistical significance of differences in proportions of serotypes in groups of patients was determined by corrected χ² test or by Fisher's exact test.

Results

Comparison of serovars causing rectal and cervical infection. The relative distribution of serovars among the cervical and rectal isolates differed significantly (figure 1). Serotype D/D' constituted 53%
Table 1. Comparison of serovars of *C. trachomatis* isolated from homosexual and bisexual men.

<table>
<thead>
<tr>
<th>Group</th>
<th>CJ</th>
<th>D/D’</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homosexual (n = 83)</td>
<td>13</td>
<td>38</td>
<td>8</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Bisexual (n = 24)</td>
<td>1</td>
<td>13</td>
<td>1</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

Comparison of homosexual and bisexual men. Of the 150 men whose rectal isolates were typed, 126 had a specific sexual preference recorded. Ninety-nine men (79%) reported exclusively homosexual contact, whereas 27 (21%) were bisexual. There were no statistically significant differences in mean or median number of sex partners or in the practice of receptive anal intercourse in the previous month. Homosexual men reported having a mean of 3.5 (± 4.0 SD) sex partners in the previous 30 days (median, 3); bisexual men had 3.4 (± 3.6 SD) partners in the same length of time (median, 2). Forty-five (45%) of 99 homosexual men and 12 (44%) of 27 bisexual men had not had receptive anal intercourse in the preceding 30 days. Although a greater proportion of bisexual men (5 [21%] of 24) had concurrent *C. trachomatis* urethral infection than did homosexual men (12 [13%] of 91), this difference was not statistically significant (P = .26, Fisher’s exact test). The most common serovars causing rectal infection in homosexual or bisexual men are shown in table 1. None of the proportions differ significantly.

Relation of serovar to clinical manifestations. All serovars of *C. trachomatis* were associated with both symptomatic and asymptomatic infections (table 2). Thirty-two (22%) of 148 men with rectal *C. trachomatis* infection were symptomatic and 54 (57%) of 94 women with cervical infection were symptomatic (P < .001). The proportion of symptomatic infections was significantly less for cervical than for rectal infections for serovars CJ, D/D’, E, and F. There was no apparent difference in the proportion of symptomatic infection for any particular serovar of rectal isolates from men but represented only 18% of cervical isolates (P < .0001). Similarly, serotype G isolates were found to represent 13% of rectal but only 3% of cervical isolates. Conversely, E was the most common serovar in cervical infections (32%) but was found in only 6% of rectal infections in men (P < .0001). Isolates of serotypes B, H, I/I’, and K represented from 2%–7% of strains in cervical isolates but were not found in the rectal isolates from men. Only two strains from homosexual men were of the LGV biovar, and both of them were L2.

To compare the distribution of serovars causing rectal infection in men and women, we typed 32 rectal isolates from women attending the clinic. The distribution of serotypes of rectal isolates from women did not differ significantly from that for cervical isolates (CJ = 3, B = 2, D/D’ = 8, E = 9, F = 7, I/I’ = 1, K = 1, mixed = 1). Although we did not isolate *C. trachomatis* serotypes B, H, I/I’ or K from the rectum of any male patient, two of 32 rectal isolates from women were serotype B, and one each was serotype I/I’ and K. None of the four women from whom serotypes B, I/I’, or K were isolated from the rectum had ano-rectal symptoms, all practiced ano-rectal receptive intercourse, and all had concurrent isolation of *C. trachomatis* from the cervix. Rectal specimens positive for *C. trachomatis* were obtained from only 11 of 314 women with cervical infection. In 10 of 11 instances, the rectal and cervical isolates were of the same serovar.

Table 2. Serotypes of *C. trachomatis* causing infection, classified by sex of affected person, site infected, presence of symptoms at infected site, and presence of concurrent *N. gonorrhoeae* at infected site.

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Rectal infection (n = 148 men)</th>
<th>Cervical infection (n = 94 women)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Symptomatic*</td>
<td>Asymptomatic</td>
</tr>
<tr>
<td>CJ</td>
<td>2, 0</td>
<td>12</td>
</tr>
<tr>
<td>D/D’</td>
<td>2, 15</td>
<td>63</td>
</tr>
<tr>
<td>E</td>
<td>2, 0</td>
<td>8</td>
</tr>
<tr>
<td>F</td>
<td>1, 2</td>
<td>17</td>
</tr>
<tr>
<td>G</td>
<td>4, 4</td>
<td>16</td>
</tr>
<tr>
<td>I/I’</td>
<td>0, 0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Data are no. positive for *N. gonorrhoeae*, no. negative for *N. gonorrhoeae*.
for either rectal or cervical infection. Eleven men with symptomatic rectal infection had concurrent \textit{N. gonorrhoeae} infection, as did 16 of the women with symptomatic cervical infection. After stratification for concurrent \textit{N. gonorrhoeae}, there were still no significant differences in proportions of either rectal or cervical infections caused by serovars C, D/D', E, and F.

\textit{Temporal variation in serovar prevalence.} Temporal variation of two types was noted during the study period: sustained trends and episodic changes in serovar distribution. $\chi^2$ Testing ($2 \times 8$ table, $df = 7$) could be performed for cervical isolates of serovars D/D', E, and F and for rectal isolates of serovar D/D' from men. The number of isolates from other serovars was insufficient for testing. No significant differences were noted in the proportions of cervical isolates of the three serovars over the course of the study. The proportion of rectal isolates of serovar D/D' decreased from 91\% to 37\% during the first four study quarters and remained relatively stable thereafter ($\chi^2 = 17.7, P = .013$). This sustained decrease contrasted with several instances in which episodes of increases of one serovar occurred among rectal isolates. The fraction of both cervical and rectal isolates of serovar G increased in the third study quarter. The proportion of rectal isolates that were serovar G was substantially increased during the sixth quarter; the proportion that were serovar CJ was substantially increased in the seventh quarter (figure 2).

\section*{Discussion}

Rectal infection with \textit{C. trachomatis} was first demonstrated in women presenting with preexisting ocular infection [10]. Subsequently, TRIC serovars of \textit{C. trachomatis} were recognized as causing rectal infection [11, 12]. Recent studies have examined the prevalence of rectal chlamydial infection in homosexual men presenting with symptoms of proctitis [13] and in unselected populations of homosexual men [14]. In these studies, LGV biovar strains have been associated with clinically severe proctocolitis. The number of isolates typed was, however, insufficient to allow clinical and epidemiological comparison with isolates from nonrectal sites or to allow assessment of the relation of specific non-LGV serovars to severity of disease.

Our data show that the prevalence of particular serotypes of \textit{C. trachomatis} among rectal isolates from homosexual and bisexual men differed from those of cervical isolates obtained from women in the same community during the same study period. Several nonexclusive hypotheses could account for these differences in serotype distribution. First, limited transmission of cervical strains to the homosexual population might allow different serotypes to predominate in each study population. We think that introduction of strains between homosexual men and heterosexual women via bisexual men is unlikely to be as important a route of transmission as is direct transmission between men. Our findings that (1) concomitant \textit{C. trachomatis} urethral infection occurred in similar proportions of homosexual men and bisexual men, (2) serovars did not differ between homosexual and bisexual men, and (3) serovars of cervical isolates differed from those of rectal isolates from men support this conclusion. The number of men in our study who described themselves as bisexual was one-fourth that of men describing themselves as homosexual, and there were no apparent differences between the serotypes of isolates obtained from bisexual or exclusively homosexual men. In our experience, men who describe themselves as bisexu-
ual are more likely to have had recent male sex partners than female sex partners; this observation agrees with recent data from Winkelstein et al. [15].

A third possible explanation for the differences in serotypes noted in this study is that certain serotypes may be less capable of survival at one or the other of the mucosal sites from which our isolates were obtained. A similar observation has been made for *N. gonorrhoeae*; certain strains are isolated more frequently from the rectums of homosexual men [16]. These strains have been shown to be resistant to fecal lipids, which occur in higher concentration in the rectum than in the vaginal-cervical environment [17]. In *C. trachomatis*, the epitopes determining serotype appear to reside on the major outer membrane protein of the organism [18], which may possess porin activity [19]. If permeability to toxic substances in the rectum is influenced by the porin activity of the major outer membrane protein, it is conceivable that serotype might indirectly reflect organism permeability. This hypothesis is suggested only by the infrequent isolation of serotypes B, H, 1/1', and K from the rectums of men both in our study and in a study of previously typed rectal strains in Seattle [3]. We isolated serotypes B, 1/1', and K from the rectums of female patients, however, a fact suggesting that the rectal environment is not always toxic to these serotypes. The fact that the women with rectal infection by these serovars had concomitant cervical infections may indicate that these serovars transiently colonize the rectum through autoinoculation, which has been suggested as the primary mechanism of rectal infection in women [20]. Autoinoculation might constantly reintroduce organisms into the rectums of women and allow isolation of serovars unable to survive the longer period between introduction of the serovar and its collection for culture from men. Our ability to reisolate *Chlamydia* from stored clinical specimens was less for rectal specimens from men than for cervical specimens, and was even less for rectal isolates from women. This fact may suggest that lower numbers of viable organisms were present in the original samples taken from the rectum, a finding that would support a role for autoinoculation in some cases of rectal infection in women.

Rectal infection with LGV serovars typically produces severe proctocolitis; infection with non-LGV serovars generally causes mild or even asymptomatic proctitis [13, 14]. To assess whether further differences in virulence between serovars causing rectal infection might occur, we evaluated the proportion of infections with each serovar associated with symptomatic proctocolitis. After excluding patients with concomitant infection by other STD agents, the number of study patients in whom we could evaluate the relation of serovar to symptomatic presentation was limited. Nevertheless, both symptomatic and asymptomatic infections were associated with each serovar, and the apparent association of each serovar with symptomatic infection was similar. Interestingly, strains of the LGV biovar were uncommon in this group of patients. Because rectal specimens for culture of *Chlamydia* were generally obtained only from those women who had rectal symptoms or who practiced receptive anal intercourse, no conclusions can be drawn from our data regarding symptoms and rectal infection in all infected women.

Chlamydial serotyping could be of great use in evaluating temporal trends of clustering of infection caused by specific strains. The number of isolates in our study was insufficient to compare the temporal occurrence of serotypes with great accuracy, but specific trends and clustering were evident. The unusual temporal distribution that we observed for rectal isolates of serovar D/D', for example, suggests that patterns of disease transmission could be evaluated in larger studies by combining active case-finding efforts with serotyping of isolates. Prospective studies of this type using larger numbers of patients would be of great value in further defining patterns of transmission of *C. trachomatis* in selected populations.

References


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