LETTERS TO THE EDITOR

Epidemiology of Chlamydia trachomatis using nested PCR

Chlamydia trachomatis is the most common agent of sexually transmitted disease in the developed countries.[1] However, its epidemiology has been little studied owing to the technical difficulty and expense of the usual typing method, which uses immunotyping.[2] This method divides strains into 15 serovars which are linked with particular types of infection (genital, trachoma, LGV) such that urogenital disease is associated with serovars D-K[3]. Recently, using the polymerase chain reaction (PCR)[4] it was shown that *C trachomatis* strains could be typed by detection of restriction fragment length polymorphism in the gene encoding the major outer membrane protein (MOMP). Although PCR amplification of *C trachomatis* plasmid DNA is more sensitive than detecting the MOMP gene[5] and has proved useful in the laboratory diagnosis of epididymitis,[6] it does not lend itself to typing as the *C trachomatis* plasmid displays no apparent variation. New developments have now resulted in a more sensitive MOMP gene detection technique using nested PCR (Frost, personal communication). We have applied the new method to groups of patients with *C trachomatis* in the Sheffield (n = 61) and the Greater Manchester areas (n = 57) for typing these strains.

All patients (comprising approximately equal numbers of males and females) attended one of three genitourinary medicine clinics (Royal Hallamshire Hospital, Sheffield; Bolton General Hospital; Withington Hospital, Manchester). Either urethral, cervical or rectal swabs were positive for *C trachomatis* by either cell culture or EIA, or both. DNA was extracted from a swab sample in 2SP medium by the method of Frost *et al.*[4] A nested PCR involving two pairs of primers (Frost, personal communication) to the constant flanking region of the MOMP was then used to detect *C trachomatis* DNA. The first primer pair was primer 1, ATGAAAAAACTCTTGAAATCGG and primer 2, GATTTTCTAGA(T,C)-TTCAT(T,C)TTG. The second primer pair was primer 3, GGGAATCCTGCTGAAC-CAAG and primer 4, AATTGCAA-(G,C)GA(A,G)ACGATTTG. All patients were positive by nested PCR. Amplified DNA samples were typed by digestion with restriction endonucleases Alu I and Msp I prior to separation on 10% polyacrylamide gels; electrophoresis was followed by silver staining.[4] Test samples were matched according to restriction endonuclease fragment patterns of control strains of the 15 serovars of *C trachomatis*.

Results showed similarities and specific differences between serovars identified in patients from the Sheffield and the Greater Manchester areas. The prevalence of D, F, G and J serovars was almost identical, although notable differences were seen for the E serovar which was more common in Sheffield, and serovar K which was found only in the Greater Manchester area (table). However, because of the small number of strains within serovars G, J and K compared in this study the results are not significant at the 5% level. Overall, these UK findings are similar to those reported elsewhere with D, E and F serovars being those commonly found in urogenital infections[2 7] together with a serovar distribution typical of western countries.

Table Serovar distribution of C trachomatis strains from Sheffield and Greater Manchester

Serovar	*Sheffield (n = 61)*	*Greater Manchester (n = 57)*
D	10 (16%)	9 (16%)
E	30 (49%)	20 (35%)
F	14 (23%)	15 (26%)
G	5 (8%)	4 (7%)
J	2 (3%)	2 (4%)
K	0 (-)	7 (12%)

$\chi^2 = 6{\cdot}53$*, 4df, $p > 0{\cdot}05$.
*Combining serovars J and K for which expected values are too small for valid chi square analysis.

We believe that this is not only the first such nested PCR study in the UK but is also the largest UK study of serovar distribution in genital infection currently available, and is of interest as a tool in epidemiological investigations and for monitoring geographical variations. Unfortunately, as urogenital *C trachomatis* infections are caused by only a small number of serovars, this method of typing, like that of serotyping, has its limitations. However, as the PCR becomes a more popular tool in the laboratory diagnosis of *C trachomatis* because of its high level of sensitivity, it is also worth noting its potential as a rapid method of typing without the need for cell culture facilities.

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1 Thompson S E, Washington A E. Epidemiology of sexually transmitted *Chlamydia trachomatis* infections. *Epidemiol Rev* 1983;5:96–123.
2 Wagenvoort J H T, Suchland R J, Stamm W E. Serovar distribution of urogenital *Chlamydia trachomatis* strains in the Netherlands. *Genitourin Med* 1988;64:159–61.
3 Grayston J T, Wang S P. New knowledge of chlamydiae and the diseases they cause. *J Infect Dis* 1975;132: 87–105.
4 Frost E H, Deslandes S, Veilleux S, *et al.* Typing of *Chlamydia trachomatis* by detection of restriction fragment length polymorphism in the gene encoding the major outer membrane protein. *J Infect Dis* 1991; 163:1103–7.
5 Mahony J B, Luinstra K E, Sellars J W, *et al.* Confirmatory polymerase chain reaction testing for *Chlamydia trachomatis* in first-void urine from asymptomatic and symptomatic men. *J Clin Microbiol* 1992; 30:2241–5.
6 Eley A, Oxley K M, Spencer R C, *et al.* Detection of *Chlamydia trachomatis* by the polymerase chain reaction in young patients with acute epididymitis. *Eur J Clin Microbiol Infect Dis* 1992;11:620–3.
7 Naher H, Petzoldt D. Serovar distribution of urogenital *C trachomatis* isolates in Germany. *Genitourin Med* 1991;67:114–6.

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Low prevalence of chlamydial endocervical infection in antenatal South Indian women

The role of genital *Chlamydia trachomatis* infection in pregnancy outcome is controversial.[1 2] There is a paucity of data from India on the prevalence of genital *C trachomatis* infection in pregnancy and therefore we determined its prevalence in pregnant women, attending a tertiary care hospital in South India and assessed the effect of such infection on the outcome of pregnancy. *C trachomatis* infection was diagnosed using Chlamydiazyme test (Abbott Laboratories). This test, reported to be both sensitive and specific,[3 4] is based on the enzyme immunoassay principle and detects the presence of chlamydia antigen in the endocervix.

Endocervical swabs were collected from 273 consecutive pregnant women attending our antenatal clinics at 26–36 weeks of gestation and processed following the manufacturer's instructions. The results were read in an Abbott Quantum II spectrophotometer using Chlamydiazyme programme. Any result equal to or greater than the cut off value calculated using the programme was considered positive.

Nine of 273 women included in the study were positive. The group studied included 100 primigravidae and 173 multigravidae. Four from the former group and five from the latter tested positive. The prevalence was higher in rural women. Six (5·9%) among 102 rural women were positive compared with three (1·8%) of 171 urban women. The results were compared with the socioeconomic status of patients assessed using a scoring system taking into account the occupation and education of the husband. None of the 44 women with lower scores had chlamydia. Three among 73 women with higher scores and six among 156 middle group were positive. None of these differences were statistically significant. In the nine women with chlamydia, the infection could not be correlated with clinical parameters like vaginal discharge, bleeding on swabbing, ectropion, dysuria or abnormal urine microscopy.

Two hundred and seven women of the study group including seven who were chlamydia antigen positive were delivered in this hospital. Association of chlamydial infection with preterm labour, premature rupture of membrane and low birthweight is shown in the table. Although there was an increased incidence of these in the infected group, the difference was not statistically significant.

The prevalence of *C trachomatis* in the population studied was 3·3%. The reported prevalence of this infection in pregnancy varies from 2–24%, usually around 7–12%.[1 5] A prevalence of 2·9% in obstetric patients was reported from China[6] which is similar to our findings.

From the present study which is the first of its kind from India, it appears that prevalence of *C trachomatis* infection is too low to play any major role in adverse pregnancy outcome in this area. This, however, is a hospital based study and a larger population based study may be necessary to assess the actual magnitude of the problem.

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1 Alexander KR and Harrison HR: Role of *Chlamydia trachomatis* in perinatal infections. *Rev Infect Dis* 1983;5: 713–19.
2 Martin DH: Chlamydial infections. *Med Clin N Amer* 1990;74:1367–87.
3 Howard LV, Coleman PF, England BJ, Herrmann JE: Evaluation of Chlamydiazyme for the detection of genital infections caused by *Chlamydia trachomatis*. *J Clin Microbiol* 1986;23:329–32.
4 Smith JW, Rogers RE, Katz BP, *et al.* Diagnosis of chlamydial infection in women attending antenatal and gynaecological clinics. *J Clin Microbiol* 1987;25:868–72.
5 Braddick MR, Ndinya-Achola JO, Mirza NB, *et al.* Towards developing a diagnostic algorithm for *C*

Table Association of endocervical chlamydia infection with pregnancy outcome

		Preterm labour		*PROM*		*LBW*	
Test	*No. studied*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Positive	7	1	(14·3)	2	(28·6)	1	(14·3)
Negative	200	7	(3·5)	35	(17·5)	23	(11·5)

PROM = premature rupture of membranes; LBW = low birthweight.