Prevalence of Chlamydia trachomatis in women attending a family planning clinic in Papua New Guinea

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Abstract
Objective—To determine the prevalence of Chlamydia trachomatis infection in women attending a family planning clinic in Papua New Guinea, in the period between April and June 1991.
Setting—The outpatient department of Obstetrics and Gynaecology of Port Moresby General Hospital, Port Moresby, Papua New Guinea, the department of Dermato-Venereology and Clinical Microbiology of the Erasmus University, Rotterdam, The Netherlands and the National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands.
Patients—A total of 254 consecutive women who attended the family planning clinic at Port Moresby General Hospital, Papua New Guinea were enrolled into this study.
Methods—Cervical infections with C. trachomatis were diagnosed using the direct immunofluorescent assay (DFA) and the polymerase chain reaction (PCR). Serum IgM and IgG antibodies directed against C. trachomatis were detected using the enzyme-linked fluorescent assay (ELFA).
Results—The prevalence of C. trachomatis was 14.6% using the PCR, 9.1% using the DFA and 17.3% when the results of the PCR and the DFA were combined. An elevated IgM titre was observed in 14.2% of the women, whereas 44.1% had an elevated IgG titre. The titres of IgM or IgG were significantly higher in women who were positive using the PCR or the DFA than in those who were negative in both the PCR and the DFA (p = 0.032 and p = 0.0046, respectively).
Conclusion—Cervical infection by C. trachomatis can be considered a major health problem in at least the studied population in Papua New Guinea. The prevalence of C. trachomatis infection is at least comparable with that in groups with a high prevalence in industrialized countries. Effective screening and treatment programmes are imperative to combat this problem.

Materials and methods
Patients The patient group consisted of 254 women who lived in the National Capital District in Papua New Guinea. The age range was 17 to 43 years (mean 25). More than 85% of the women were married. Approximately 30% of the study population did not have any form of formal education. All women visited the outpatient Department of Obstetrics and Gynaecology of Port Moresby General Hospital, between April and June 1991. Cervical swabs were obtained from all patients to establish the presence of C. trachomatis using culture. Owing to import problems, however, the swab samples were thawed and we decided to perform PCR rather than culture. At the same visit also cervical smears were obtained for DFA and a blood sample was taken from each patient to determine IgM and IgG antibody titres against C. trachomatis. After drying, smears intended for DFA, were fixed. At the end of the study, the swabs, fixed smears and blood samples were sent by air mail to the departments of Dermato-Venereology and Clinical Microbiology, University Hospital Dijkzigt, Erasmus University, Rotterdam, The Netherlands.

Keywords: Chlamydia trachomatis; Family planning; Papua New Guinea

Introduction Sexually transmitted diseases (STDs) such as gonorrhoea, syphilis and infections with chlamydiae are a major health problem in developing countries. In contrast to what is observed in industrialised countries, the prevalence of STDs in developing countries appears to be increasing. The prevalence of genital Chlamydia trachomatis infections in the USA, in a college student population, was approximately 5%. In developing countries, the prevalence varies per individual country and within each population. It is observed to vary from 6-3% in women attending a Kenyan family planning clinic to 44% in pregnant women in El Salvador. The frequent occurrence of complications (such as pelvic inflammatory disease and neonatal conjunctivitis) as a direct consequence of this high prevalence is a major health problem in developing countries. In Papua New Guinea, 17-7% of the women who at an antenatal clinic were found to be infected with C. trachomatis. In our study, the prevalence of genital C. trachomatis infections was investigated in women attending the family planning clinic at Port Moresby General Hospital, Port Moresby, Papua New Guinea. The polymerase chain reaction (PCR) and the direct fluorescent assay (DFA) were used to establish current infections. At the same time, IgM and IgG antibody titres were determined in order to obtain information about the spread of C. trachomatis infection.
Microbiology, University Hospital Dijkzigt and Erasmus University Rotterdam and to the National Institute of Public Health and Environmental Protection (RIVM) in Bilthoven, for further processing.

Cultures to determine the presence of *N. gonorrhoeae* in the cervix were not performed because the appropriate culture media were not available at the laboratory of Port Moresby General Hospital.

**Polymerase Chain Reaction (PCR)**

Swabs for detecting *C. trachomatis* using the PCR were frozen at −70°C in 1·5 ml 20 mM phosphate buffer containing 0·2 M sucrose (Merck, Darmstadt, Germany), 49 mM glutamine (Sigma, St. Louis, MO, USA), 10% FCS, 18 μg/ml gentamicin, 23 μg/ml vancomycin and 2·5 μg/ml amphotericin B (SPG). The PCR was performed according to the method described by Claas et al. DNA was extracted from 0·5 ml of the sample from each patient. Two sets of oligonucleotide primers were used. The first set (R1 = GTGGATAGCTT-CAACCCTAT, R2 = TATCTGTCTTT-GCGGAAAAAC) was derived from 16S rRNA gene sequences of *C. psittaci* and generated a 208-bp amplified product with all four Chlamydia species. The second set (T1 = GGACAAATCGTATCTCGG, T2 = GAAAACACTCTAGCGT) was derived from sequences of the common endogenous plasmid of *C. trachomatis* and generated a 517-bp product with all known *C. trachomatis* serovars. After 40 cycles, the amplification product was analysed on a 2% agarose gel.

**Direct Immunofluorescent Assay (DFA)**

*C. trachomatis* elementary bodies (EBs) were detected using the DFA as follows. In the Port Moresby General Hospital, a smear of the sample from the cervix was prepared on a glass slide. After drying, it was fixed in methanol. After postage to the National Institute of Public Health and Environmental Protection, the preparation was then stained with FITC-conjugated anti *C. trachomatis* monoclonal antibody (Microtrak, Syva Co., Palo Alto, Calif.) and examined at a magnification of ×500 using a fluorescent microscope. The presence of 5 or more EBs was regarded as positive.

**Enzyme-Linked Fluorescent Assay (ELFA)**

Specific IgM and IgG antibodies to *C. trachomatis* were determined according to the method described previously. Wells of PVC microtitre plates were coated with 50 μl of a 10 μg/ml elementary bodies (EB) suspension of the *C. trachomatis* serotype E (strain UW-5/cx), F (strain 1C-Cal3), H (strain UW-4/Cx), I (strain UW-12/Ur), J (strain UW-36/Cx) and LGV2 (strain 343/Blu) in 0·1 M carbonate/bicarbonate buffer (pH 9·6). After incubation at 4°C for 16 h, the unoccupied binding-sites were blocked with PBS containing 2% BSA. The plates were subsequently rinsed with PBS containing 1% BSA and 0·05% Tween-20 (PBST) and incubated with 50 μl diluted test serum (1:250 for IgM determination and 1:1000 for IgG determination) at 22°C for 45 min. The plates were then rinsed with PBST and incubated at 22°C for 45 min with either 50 μl of 1:8000 dilution of anti-human IgG biotin (Boehringer, Mannheim, Germany) or with 50 μl of 1:20 dilution of anti-human IgM which had been coupled to β-galactosidase. The plates were then rinsed with PBST and those for IgG titre determination incubated for a further 45 min at 22°C after addition of 50 μl of 1:4000 dilution of streptavidin-β-galactosidase (Boehringer) and subsequently rinsed with PBST. The final step consisted of a 1 h incubation at 37°C with 100 μl of 0·25 mg/ml 4-methylumbelliferyl β-D-galactoside (Sigma, St. Louis, MO, USA) in a buffer containing 44 mM K2HPO4, 55 mM KH2PO4, 4 mM MgCl2 (pH 7·6). The reaction was stopped by adding 100 μl of 1 M Na2CO3. Fluorescence was measured at 480 nm using Fluoroscant 1 (Flow Laboratory, Irvine, Scotland). The negative cut-off points for IgG and IgM were determined as described previously.

**Results**

While only 12% of the women were complaining about vaginal discharge, vaginal infection was diagnosed in considerably more women. Bacterial vaginosis and trichomoniasis were especially prevalent (25 and 22% respectively). We did not find an association between gynaecological complaints (namely abdominal pain and/or vaginal discharge) and the presence of *C. trachomatis* infection.

The prevalence of cervical *C. trachomatis* infections determined using PCR and DFA is shown in the table. The number of patients who were positive using PCR as the method for detection did not differ significantly from the number who were positive using DFA (p = 0·073). Although the majority of patients who were positive using DFA were also positive using PCR (69%), the overlap was not large enough to justify the use of the PCR as the sole method for detection.

Increased IgM titres (≥1/250) were observed in the sera of 36 (14·2%) patients. In 10 of these 36 IgM positive patients, cervical *C. trachomatis* infection was established using PCR, whereas 26 patients were negative using PCR. There were significantly more patients with an increased IgM titre in the PCR-positive group than in the PCR-negative group (p = 0·014). The majority of the patients who were positive using PCR and DFA had an increased IgM titre as compared with those who were negative using PCR and DFA (p = 0·036). This difference was not sig-

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**Prevalence of Chlamydia trachomatis as measured by the polymerase chain reaction (PCR) and/or the direct immunofluorescent assay (DFA)**

<table>
<thead>
<tr>
<th>Detection method</th>
<th>Positive patients</th>
<th>Negative patients</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR</td>
<td>37</td>
<td>217</td>
<td>14·6</td>
</tr>
<tr>
<td>DFA</td>
<td>23</td>
<td>231</td>
<td>9·1</td>
</tr>
<tr>
<td>PCR and DFA</td>
<td>16</td>
<td>238</td>
<td>6·3</td>
</tr>
<tr>
<td>PCR or DFA</td>
<td>44</td>
<td>210</td>
<td>17·3</td>
</tr>
</tbody>
</table>

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sificant when comparing IgM titres and results of DFA only (p = 0·11).

Increased IgG (≥1/1000) titres were observed in the sera of 112 (44·1%) patients. In 26 of these 112 patients, cervical C trachomatis infection was established using PCR, whereas 86 patients who had an increased IgG titre were negative using PCR. The IgG titres were significantly higher in the PCR-positive group than in the PCR-negative group (p = 0·0006). The number of patients with an increased IgG titre in the PCR- and the DFA-positive group was higher than that in the PCR- and the DFA-negative group (p = 0·0046). This difference was not statistically significant when comparing IgG titres and results of DFA only (p = 0·27).

Discussion
In this study, using PCR and DFA, a cervical C trachomatis infection was established in respectively 14·6% and 9·1% of the women who attended the family planning clinic of Port Moresby General Hospital. Since there is a good correlation between the results of tissue culture and that of PCR9 and since there was a somewhat less agreement between DFA and tissue culture,10 the prevalence observed using PCR appeared to be more reliable. Since only 69·9% of the patients who were positive using DFA were also positive using PCR, in this patient group it appeared to be insufficient to use only PCR for reliable diagnostics. In part these discordant results of PCR and DFA can be explained by assuming a rather low number of elementary bodies in some of the patients. Because transport of swabs and smears was delayed owing to administrative problems, the transport medium in the swab-containing tubes was thawed on arrival. This certainly has had adverse effects on PCR sensitivity. Because DFA smears were fixed prior to transport, sensitivity of DFA was not, or was considerably less, affected by the transport delay. So in our opinion the positive results of both PCR and DFA, for reasons described above, should be combined to give the best estimation of the extent of urogenital C trachomatis infection in the study population, altogether being 17·3%. This corroborated the prevalence that was observed previously, using DFA as the method for detecting C trachomatis in a group of pregnant women in Papua New Guinea.5 Such high prevalences of genital C. trachomatis infections have also been reported from other developing countries11-13. Owing to limited resources, however, PCR as well as DFA or culture are not suitable for screening purposes in developing countries. Relatively cheap tests that give results within a hour are theoretically more suitable for use in developing countries. Because of the often low sensitivity of such tests, if compared with culture,14 further research is needed, before use in developing countries can be advocated.

The composition of the patient group studied was comparable with that of a sexually active female population aged between 15 and 40 years in the industrialised world. The prevalence of IgM (14·2%) and IgG (44·1%) antibodies against C trachomatis in the patient group studied was identical to that in a group of patients with a high prevalence of C trachomatis infections in the western world.15 Using the combination of PCR and DFA results as the best estimation of the amount of genital C. trachomatis infections in the population studied, the sensitivity and the specificity of an increased titre of IgM antibodies against C trachomatis were observed to be 25·0% and 88·1% respectively. Similarly, the sensitivity and the specificity of an increased IgG titre were observed to be 63·6% and 60·0% respectively. Similar figures were also previously reported from Rotterdam in a group of women with a high prevalence of C trachomatis antibodies.

Logistically, it was not possible to determine sero-conversions in the group of patients in Papua New Guinea. As a direct consequence and because of the observation that IgG antibodies can be demonstrated in serum for many years,16 the detection of IgM and/or IgG antibodies against C. trachomatis was unsuitable for establishing acute infections. Also, trachoma is endemic in Papua New Guinea. Because C trachomatis serovars causing trachoma and urogenital infections are indistinguishable in the serological test used, the percentage of the population with an increased titre of IgG antibodies against C trachomatis is only useful as an epidemiological indicator of the extent to which C trachomatis had spread in the population. As such, it was established that approximately half of the women studied had previously been in contact with C trachomatis.

It is concluded that cervical C trachomatis infections are widespread in Papua New Guinea and that there is a definite need for screening women who visit secondary and tertiary health care facilities, especially those of childbearing age.

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Dr B Tank is thanked for correcting the English text.

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