Genital chlamydial infection among women in Nicaragua: validity of direct fluorescent antibody testing, prevalence, risk factors and clinical manifestations

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Objective: To validate the performance of a direct fluorescence antibody (DFA) test and to determine the prevalence, risk factors and clinical manifestations of cervical chlamydia infection in different groups of women in Nicaragua.

Study population: 926 women, 863 routine clinic attenders (mean age 27 years) and 63 sex workers (mean age 25 years) attending health centres in León, Corinto, Matagalpa and Bluefields.

Methods: Cervical specimens were examined using the Syva MicroTrak test system with a cut-off of 10 or more elementary bodies (EBs). The DFA results were validated by a one-step polymerase chain reaction (PCR) assay. Discordant results were further examined in nested PCR assays directed at two different target genes. An interviewer-administered questionnaire and a standard gynaecological examination were completed.

Results: Sensitivity of DFA was 80.1%, specificity 98.3%, and positive and negative predictive values 62.5% and 99.3%, respectively. Values were lower in locations where samples thawed because of electricity breaks and higher among sex workers. The majority of discordant results was confirmed as positive in nested PCR assays. Prevalence of cervical chlamydia infection based on positivity in DFA and/or PCR ranged from 2% among routine clinic attenders aged 35 years or older, to 8% among adolescent clinic attenders, and to 14% among sex workers. Among routine clinic attenders, young age (odds ratio [OR] 3.6, 95% confidence intervals [95% CI] 1.4–9.9) for women aged 15–19 years as compared with 1 in women 25 years of age or older) and use of oral contraceptives (OR 4.0, 95% CI 1.7–9.6) were the only statistically significant risk factors identified in multivariate logistic regression analysis. Presence of mucopurulent cervical discharge (OR 5.9, 95% CI 3.0–11.5) and presence of ectropion (OR 2.6, 95% CI 1.6–6.5) were the clinical signs independently associated with infection.

Conclusions: Our results indicate that the DFA test was sensitive and specific while the performance of the PCR assay depends on adequate storage of samples. Genital C trachomatis infection is a common health problem among women in Nicaragua. The wide implementation of syndromic STD management algorithms together with health education programmes aimed at young people is the most promising approach to control STD in Nicaragua.

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Keywords: Chlamydia trachomatis; women; direct immunofluorescence testing; polymerase chain reaction; risk factors; Nicaragua

Introduction

Infection by Chlamydia trachomatis is a common sexually transmitted disease (STD) both in industrialised and developing countries. In women, the organism can cause urethritis and cervicitis, but up to 70% of infections are asymptomatic. Pelvic inflammatory disease—endometritis and salpingitis—may develop if the infection remains untreated and can lead to infertility, ectopic pregnancy and chronic abdominal pain. Neonates delivered vaginally from infected mothers may contract chlamydial conjunctivitis or pneumonia. In men, infection can cause urethritis and epididymitis, but 25% to 50% of infected men remain asymptomatic.

The awareness of genital chlamydial infections in developing countries is still limited. At district level, diagnostic facilities are generally unavailable. In larger centres, diagnosis is predominantly based on first generation enzyme immuno assays (EIA) which are known to have suboptimal sensitivities, or on culture with outdated staining techniques such as Giemsa or iodine. More recently, the introduction of direct fluorescent antibody (DFA) tests has improved the detection of the organism in a number of settings. Together with other non-culture based tests, the DFA method is valuable for examination of specimens that have lost viable chlamydia through prolonged transport or inadequate storage. The DFA test can be very sensitive; however, its performance is highly observer-dependent.

Validation of DFA findings is therefore required for clinical and epidemiological research both in industrialised and developing countries. Polymerase chain reaction (PCR) is a non-culture test system whose sensitivity and specificity for detection of genital chlamydia infection is comparable or even better than cell culture. It is therefore well suited for evalua-
tion of DFA in a less developed country lacking the facilities for culture.

Genital chlamydia infections have not been studied so far in Nicaragua. Data for other Central American countries are also scarce. In order to determine prevalence and risk factors of genital *C. trachomatis* infections, we conducted a study among women attending routine clinics and sex workers attending special clinics in health centres in different regions of Nicaragua. Here we describe the performance of the locally performed DFA tests in comparison with PCR analysis in a Swedish laboratory, and the prevalence, risk factors and clinical presentation of the infection among Nicaraguan women.

**Materials and methods**

**Study population**

Women aged 15 to 45 years attending gynaecological, cervical screening (Papanicolaou smear), antenatal, or family planning clinics located at public health centres in the municipalities of León, Bluefields and Matagalpa, and female sex workers from the ports of Corinto and Bluefields participated in this study. The study locations, shown on the map (Fig), cover the densely populated Pacific coast (León, Corinto), the central mountainous region (Matagalpa) and the ethnically diverse Atlantic coast (Bluefields). The study started in León in March 1992 and concluded in Matagalpa in June 1993. In each location women were recruited during a period of about four months. For health centre attenders, the sample size envisaged at each study site was around 300 women. Sex workers were examined at special clinics which were set up within the framework of an STD and acquired immunodeficiency syndrome (AIDS) prevention programme in Corinto and Bluefields.8 The women were from urban settings except for Matagalpa where exclusively women from coffee growing farms participated. Consecutive women attending the clinics were included after giving verbal consent. An interviewer-administered standardised questionnaire and a standard gynaecological examination was then completed and specimens were collected. This included two cervical swabs for the diagnosis of chlamydia and gonorrhoea, and a vaginal swab for a wet mount preparation. All women received free treatment and counselling if a sexually transmitted infection was diagnosed. Women with positive DFA results were traced by a nurse for this purpose as soon as the result became available.

**Specimen collection and DFA testing**

A non-lubricated speculum was passed and the cervix cleaned with a cotton-tipped swab. An endocervical specimen was then taken with a non-toxic calcium alginate swab (ENT, Medical Wire & Co, UK) and rolled onto a 6-mm slide well before placing it into a cryotube with sucrose phosphate buffered saline transport media for testing by PCR. Slides for DFA tests were fixed in methanol and stored up to 3 weeks at +8°C before staining with monoclonal antibodies according to the instructions of the manufacturer (MicroTrak, Syva, USA). A slide was considered positive if 10 or more EBs were detected upon examination. All slides were examined by a single laboratory technician who was trained by one of us (BH). A standard Zeiss microscope fitted with a fluorescence condenser type IVFI was used with 400 × magnification for reading. Specimens were considered inadequate if they contained less than 10 epithelial cells, or if the smear was too thick for individual cells to be brought into focus.

**Storage and transport**

The swabs for PCR analysis were kept in a cool box at +8°C up to six hours before being frozen at −70°C (León, Corinto) or −20°C (Bluefields, Matagalpa). In Matagalpa and Bluefields, samples thawed and were refrozen on up to three occasions following electricity cuts. After completion of the study, samples were transported by air in a cool box at +8°C until being frozen at −20°C on arrival in Uppsala.

**PCR analysis**

Nucleic acid for one-step PCR analysis was prepared from 200 µl volume of each specimen, centrifuged at 12,000 g for 20 minutes and suspended in 20 µl lysis buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 1% Triton X-100 and 200 µg proteinase K per ml). After incubation at 60°C for one hour and 95°C for 10 minutes, PCR amplification was performed.

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Map of Nicaragua indicating the four study locations and the capital Managua. Routine clinic attenders were examined in León, Matagalpa and Bluefields and sex workers in Corinto and Bluefields.
according to the method of Ossewaarde et al. on a 2 μl lysate in 20 μl reaction mixture. The latter contained 0.4 μM oligonucleotide primer and 1.0 U of Taq-polymerase (Boehringer-Mannheim). The 493 bp fragment from the cryptic C trachomatis plasmid was detected after electrophoresis on 1% agarose gel stained with ethidium bromide under UV-light. Two positive controls with 10² and 10⁴ EBs, respectively, and a negative control were included in each run. The laboratory technician performing the one-step PCR tests was blind to the DFA results.

Samples positive by DFA but negative in the one-step PCR were further analysed by a nested PCR assay including the C trachomatis specific inner primer pair Pd3 (5’GGCC-TCTAAAACGGCTAGGCCG3’) and Pd4 (5’TTCGTCTAATCTTACGGATCCCTTG3’). The selected set amplified a fragment of 259 bp from nucleotide 6862 to nucleotide 7121 of the plasmid of serovar L2.10 After preparing a new nucleic acid sample and amplification with the outer primer pair Pd1 and Pd2, 1 μl was transferred to 50 μl of a new reaction mixture containing 0.4 μM oligonucleotide Pd3 and Pd4, 200 μM nucleotides, 2.0 mM MgCl₂, 0.25 mM Tris-HCl (pH 8.3), 50 mM KCl, and 1.0 U Taq-polymerase (Boehringer-Mannheim). The samples were subjected to a denaturation step at 95°C for 3 minutes in a Perkin-Elmer 9600 thermocycler followed by 30 cycles of amplification, consisting of 30 seconds at 95°C, 1 second at 60°C and 45 seconds at 72°C. Amplification was completed by extension at 72°C for 5 minutes. To confirm samples positive in both DFA and the nested plasmid PCR analysis and exclude false positive cases, a second nested PCR method was introduced by using the cysteine rich outer membrane protein (CrP) gene as the target. This PCR assay was performed according to the amplification previously described by Wahlberg et al.11 but amplification products were detected by electrophoresis as described above. Samples negative in DFA testing but positive in the one-step PCR were also tested in the nested PCR assay directed at the CrP gene. Inhibitory components were analysed in a subset of 168 clinical samples by introducing a weak positive control (10² EBs) of C trachomatis DNA in the reaction mixture of 20 μl. Amplicon contamination was surveyed by inclusion of a negative control after each fifth clinical sample.

Statistical analysis

Only women who had both DFA and PCR exams done and whose DFA sample was considered adequate by the microscopist were included in the analysis. Sensitivity, specificity and positive and negative predictive values were then calculated, taking the one-step PCR results as the gold standard. For the assessment of the prevalence of genital chlamydial infections, women positive in only one test were considered to be infected. Comparisons between groups employed chi-square tests (with continuity correction when appropriate) and student’s t tests. Multivariate logistic regression models were also calculated. Results are presented as percentages, means and odds ratios with 95% confidence intervals.

Results

In the routine outpatient clinics, a total of 1038 women were examined but only 951 (92%) had their sample analysed both by DFA and PCR. Among the latter, an adequate DFA sample had been collected in 863 women (90%). In addition, a total of 67 female sex workers were examined at the special clinics, 100% of whom had both analyses done. The quality of the samples was inadequate in four cases. In total, 863 women attending routine clinics (259 from Leon, 267 from rural Matagalpa and 337 from Bluefields) and 63 female sex workers (42 from Corinto and 21 from Bluefields) were included in the present analysis, for a total of 926 women. Mean age was 27.3 years among routine clinic attenders as compared with 25.6 among sex workers. At the routine clinics the majority of women were either married (30%) or lived in free union (53%) whereas most of sex workers (79%) were single. Eighty-eight percent of routine clinic attenders had given birth (mean 3.7 births) as compared to 81% among sex workers (mean 3.0 births). Twenty-one percent of routine clinic attenders were illiterate as compared to six percent among sex workers.

Overall, 40 (4.3%) slides were positive in the DFA examination as defined by the detection of 10 or more EBs. Furthermore, there were 55 (5.9%) slides with 1–4 EBs, and five (0.5%) with 5–9 EBs. Fifteen DFA positives were not confirmed by one-step PCR analysis while 6 DFA negatives had a positive one-step PCR result, for an overall sensitivity of 80.1%, a specificity of 98.3% and positive and negative predictive values of 62.5% and 99.3%, respectively. All five DFA samples with less than 10 EBs were negative both in one-step PCR assay and nested PCR assays.

As shown in table 1, performance of DFA among routine clinic attenders appears to have been better in Leon as compared with Bluefields and Matagalpa. In Matagalpa and Bluefields samples thawed (and were refrozen) twice and three times respectively following electricity breaks. Comparing Leon with the other two locations, sensitivity was 88.9% versus 71.4% (p = 0.06), specificity 99.6% versus 97.8% (p = 0.009), positive predictive value 88.9% versus 43.5% (p = 0.000), and negative predictive value 99.6% versus 99.3% (p = 0.09). Performance among sex workers was also

Table 1  Performance of DFA testing for genital Clamydia infection compared to one-step polymerase chain reaction analysis among different female populations in Nicaragua

<table>
<thead>
<tr>
<th>Population</th>
<th>% sensitivity</th>
<th>% specificity</th>
<th>% PPV</th>
<th>% NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Routine clinic attenders</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leon</td>
<td>89.9 (8/9)</td>
<td>96 (249/250)</td>
<td>89.9 (8/9)</td>
<td>96 (249/250)</td>
</tr>
<tr>
<td>Bluefields</td>
<td>72.7 (8/11)</td>
<td>97.5 (318/321)</td>
<td>99.0 (55/56)</td>
<td>99.0 (318/321)</td>
</tr>
<tr>
<td>Matagalpa</td>
<td>66.7 (2/3)</td>
<td>98.1 (259/264)</td>
<td>25.6 (2/9)</td>
<td>25.6 (259/264)</td>
</tr>
<tr>
<td>Combined</td>
<td>75.6 (15/18)</td>
<td>98.3 (826/831)</td>
<td>56.3 (18/32)</td>
<td>99.4 (826/831)</td>
</tr>
<tr>
<td>Sex workers</td>
<td>97.9 (7/8)</td>
<td>98.2 (54/55)</td>
<td>97.5 (7/8)</td>
<td>98.2 (54/55)</td>
</tr>
<tr>
<td>All women</td>
<td>90.1 (25/31)</td>
<td>98.3 (800/804)</td>
<td>62.5 (24/40)</td>
<td>99.3 (800/804)</td>
</tr>
</tbody>
</table>

Percentage (numbers). PPV: positive predictive value. NPV: negative predictive value.

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better than among the routine clinic attenders examined outside Leon.

Fourteen out of 15 DFA positive, but one-step PCR negative, tests could be further examined in nested PCR assays. One sample had to be excluded because of insufficient material. Ten out of 14 were positive while the other four samples remained negative in both nested assays. All six DFA negative but one-step PCR positives were confirmed as positive in the nested PCR assay directed at the Crp gene. Therefore, 16 out of 20 discordant samples (80%) were confirmed as positive in nested assays. Taking these results into account would considerably improve the figures mentioned above. Overall, specificity would increase from 98.3% to 99.6%, and the positive predictive value from 62.5% to 89.7%. However, samples negative in both DFA and one-step PCR were not further analysed in nested PCR assays. Analysis of inhibiting components for the PCR assay showed inhibition in 9 of 168 (5.4%) samples. Repeated testing of these 9 samples using a 100 μl reaction mixture revealed the inhibition to be dilution dependant since 8 samples resulted in a 493 bp amplicon.

Prevalence of cervical chlamydial infection based on positivity in DFA and/or PCR was 4.3% among women examined in routine clinics as compared with 14.3% among sex workers (p = 0.005). Risk factors for infection were further examined among routine clinic attenders. As shown in table 2, young age and use of oral contraception were the only statistically significant predictors for infection. Both in univariate and multivariate analysis, marital status, clinic location, clinic type, use of an intrauterine device, and self-reported history of STD, literacy and housing conditions did not significantly influence the risk of infection (p > 0.05). Women were also asked whether or not they had been with the same sexual partner during the last three months; however, 16% percent refused to answer this question. Among those who did answer there was no statistically significant difference between the small minority reporting more than one partner and those reporting a stable partner.

The association of symptoms and signs with the probability of chlamydial infection was also examined. Sixty-three percent of women complained of vaginal discharge (64% of routine clinic attenders as compared with 49% of sex workers, p = 0.03), 55% of abdominal pain (56% versus 42%, p = 0.04) and 38% of itching (39% versus 27%, p = 0.07). None of these symptoms were predictive of chlamydial infection. On examination, 69% were found to have abnormal vaginal discharge, 16% had mucopurulent discharge from the cervix, 33% oedema of the cervix, 19% bled when taking the cervical specimen (cervical frailty) and 6% had some degree of ectropion. Prevalence of clinical signs was similar among routine clinic attenders and sex workers except for ectropion which was more frequent among sex workers (27% as compared with 4% among routine clinic attenders, p = 0.0001). In univariate analysis, mucopurulent cervical discharge (p = 0.0001), bairness of the cervix (p = 0.03) and ectropion (p = 0.01) were all associated with the probability of infection. In multivariate analysis, however, only the presence of mucopus and ectropion were independently predictive of chlamydial infection (table 3).

### Table 2 Risk factors for genital Chlamydia infection among 863 women attending routine clinics

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>% Prevalence (numbers)</th>
<th>Odds ratio (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1</td>
<td>8-0 (10/125)</td>
<td>3-6 (1-4-8-9)</td>
<td>0-01</td>
</tr>
<tr>
<td>2-5</td>
<td>6-7 (16/239)</td>
<td>3-4 (1-1-5-9)</td>
<td>1-0</td>
</tr>
<tr>
<td>≥ 25</td>
<td>2-2 (11/499)</td>
<td>1-0</td>
<td>0-3</td>
</tr>
</tbody>
</table>

5-22: 0.04–0.07

<table>
<thead>
<tr>
<th>Marital status</th>
<th>% Prevalence (numbers)</th>
<th>Odds ratio (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single</td>
<td>4-9 (7/143)</td>
<td>2-2 (0-7-4-5)</td>
<td>0-3</td>
</tr>
<tr>
<td>Free union</td>
<td>5-1 (23/454)</td>
<td>2-4 (0-7-4-5)</td>
<td>1-0</td>
</tr>
<tr>
<td>Married</td>
<td>2-7 (7/256)</td>
<td>1-0</td>
<td>0-3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clinical location</th>
<th>% Prevalence (numbers)</th>
<th>Odds ratio (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bluefields</td>
<td>5-3 (18/337)</td>
<td>1-5 (0-6-3-0)</td>
<td>0-02</td>
</tr>
<tr>
<td>Leon</td>
<td>3-0 (10/259)</td>
<td>0-8 (0-3-2-4)</td>
<td>0-6</td>
</tr>
<tr>
<td>Rural Matagalpa</td>
<td>3-4 (9/267)</td>
<td>1-0</td>
<td>0-9</td>
</tr>
</tbody>
</table>

### Table 3 Clinical signs associated with genital Chlamydia infection

<table>
<thead>
<tr>
<th>% Prevalence (numbers)</th>
<th>Odds ratio (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaginal discharge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>5-5 (35/639)</td>
<td>1-1 (0-5-2-1)</td>
</tr>
<tr>
<td>Absent</td>
<td>3-9 (11/282)</td>
<td>1-0</td>
</tr>
</tbody>
</table>

| Mucopus in cervix      |                     |    |
| Present                | 1-5 (23/151)        | 5-9 (3-0-11-5)      | 0-01 |
| Absent                 | 3-0 (23/771)        | 1-0                 | 0-01 |

| Oedema of cervix       |                     |    |
| Present                | 5-5 (17/308)        | 0-5 (0-3-1-1)       | 0-3 |
| Absent                 | 4-7 (29/614)        | 1-0                 | 0-04 |

| Faleness of cervix     |                     |    |
| Present                | 8-4 (15/178)        | 1-5 (0-7-3-2)       | 0-3 |
| Absent                 | 4-2 (31/744)        | 1-0                 | 0-04 |

| Ectropion              |                     |    |
| Present                | 13-5 (7/52)         | 2-6 (1-1-6-5)       | 0-0001|
| Absent                 | 4-5 (39/874)        | 1-0                 | 0-0001|

Analysis based on 863 routine clinic attenders and 63 sex workers. Data missing in up to five weeks (95% confidence interval. Odds ratios and p values from multivariate logistic regression model adjusting for all variables listed.

### Discussion

The advent of nucleic acid amplification techniques for the diagnosis of C trachomatis infection has facilitated evaluation of antigen detection methods in situations when cell culture is not a feasible option. In ideal circumstances, PCR tests have been shown to be
equally or more sensitive and specific than cell culture.\textsuperscript{7,8} In the present study in Nicaragua, the DFA method had a sensitivity of 80% and a specificity of 98% when compared with a one-step PCR assay. Previous studies, using cell culture as the gold standard, have reported sensitivities between 60% and 81%, and specificities in the range of 82% to 99%.\textsuperscript{12-15} However, in highly trained expert hands with a cut-off of just one elementary body, DFA testing can be more sensitive than culture.\textsuperscript{16}

Taken together, these data illustrate the crucial role of the observer in DFA testing and indicate the absence of a definite gold standard for \textit{C trachomatis}. In the present study a laboratory technician without previous experience in fluorescence microscopy was carefully trained. Although comparing well to the results from other studies, her performance may well be underestimated when DFA results are exclusively contrasted with one-step PCR testing. A surprisingly high number of DFA positive cases with a negative one-step PCR test was found predominantly in study locations where inadequate storage led to repeated thawing of samples. Repeated thawing and freezing is considered to be detrimental for the stability of DNA sequences.\textsuperscript{13} Unfortunately, dry ice or liquid nitrogen was not available at the time. The amount of intact DNA still present when tested by PCR may thus have been insufficient in a number of samples from these locations. Sensitivity and specificity was also higher among sex workers than among routine clinic attenders. As a consequence of repeated exposure, the antigen may have been present in higher concentrations among the latter. This interpretation is supported by the results from nested PCR assays whose sensitivity is enhanced compared with one-step assays. With the nested assays, ten out of 14 DFA positive results were confirmed, leading to a considerably improved sensitivity and positive predictive value of the DFA test. Overall, 80% of discordant samples were confirmed as positive in the nested assays. These results should, however, be interpreted with caution because these assays were exclusively performed on discordant samples. Nested PCR systems suffer from the risk of cross-contamination leading to false positive results. This is, however, less likely in the present study since the two nested PCR assays used different target genes but gave completely concordant results. Taking these results into account would nevertheless overestimate the specificity and the positive predictive value of the DFA test.

In principle, DFA testing thus appears to be an adequate technique for the diagnosis of \textit{Chlamydia trachomatis} in Nicaragua and other less developed countries. In a research context, validation by PCR or culture will often be necessary. In many settings these tests will have to be performed in an industrialised country. PCR analysis of frozen samples is valuable; however, much attention must be given to adequate storage and transport of samples in order to prevent thawing. In a recent study in Cape Verde, we found analysis of dried samples that can be kept at room temperature to be superior, allowing reliable detection of chlamydial antigen after more than 18 months of storage.\textsuperscript{18}

It is noteworthy that the prevalence found in our study is considerably lower than that reported for urban populations in the neighbouring countries Honduras and El Salvador.\textsuperscript{19,20} Using a commercial EIA kit (Pharmacia Chlamydia EIA Test, Pharmacia, Sweden) a prevalence of 44% was reported for pregnant women in El Salvador, as compared with 28% for Salvadoran women with cervicitis and 5% for asymptomatic women. These results, however, are quite unusual, indicating that there were problems with the test system which led to false positive results. Indeed, in their study in Honduras,\textsuperscript{21} the same group reported that about 20% of samples had to be excluded because of high absorbance values in negative controls. Unfortunately, no systematic validation of these results using a different test system was performed. On the other hand, our results are similar to the findings from studies conducted in Panama, the Netherlands Antilles and Chile. The prevalence among women at high risk of infection in urban Panama ranged from 2% to 8%.\textsuperscript{22} Sixteen percent of sex workers from Santiago de Chile and 5% of women attending gynaecological clinics on the Netherlands Antilles were found to be chlamydia positive.\textsuperscript{23,24}

Our results will tend to underestimate the prevalence of genital chlamydia infection among Nicaraguan women. It has been stated that "the more you look" (for genital chlamydia infection) "the more you find".\textsuperscript{10} For example, by testing both samples from the cervix and the urethra, the yield is increased by 10% to 20%. Furthermore, as discussed above, it is clear that neither method used in this study was 100% sensitive. Although a lower cut-off value were confirmed, leading to a considerable improvement in comparison with the one-step PCR, it is very likely that at least some of the samples with less than ten EBs stemmed from women with cervical infection.

Young age, use of oral contraceptives and commercial sex work were the risk factors for chlamydial infection identified in this study. Multiple sexual partners, unmarried status and low socio-economic position have also been shown to predict infection.\textsuperscript{2} In the present study, a substantial proportion of routine clinic attenders refused to answer the question regarding multiple sexual partners and no statistically significant association with their marital status was evident. Both findings may be related to the concept of machismo which is central in defining sexual relations in Nicaragua and other Latin countries.\textsuperscript{25} In men, machismo values encourage early sexual initiation and multiple sexual conquests, while repressing sexual autonomy in women. Marital status of the woman may thus be an imprecise proxy measure for the sexual behaviour of her husband. The study population was relatively homogeneous with regard to socio-economic status. Women of higher social standing generally do not attend public
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health centres but consult private clinics. This may explain the absence of an association with socio-economic factors. Our study confirms the clinical signs that are generally found to be associated with chlamydial infection of the cervix.2 The strongest association was found with mucopurulent discharge from the cervix, thus supporting the use of this symptom in the syndromic algorithm for the management of vaginal discharge that is recommended by the World Health Organisation (WHO).26

Our findings have important implications for STD control in Nicaragua. Both among women attending routine care and among female sex workers, the prevalence of chlamydial infection is considerably higher than gonorrhoea.27 However, the importance of chlamydial infections is not yet widely appreciated. Benzathine penicillin is still frequently used as the exclusive treatment in case of mucopurulent cervicitis although this is of questionable value for gonorrhoea and ineffective for chlamydial infection.28–29 The high prevalence among young women would justify generalised screening in antenatal and other routine clinics; however, considering the lack of trained personnel, and the limited laboratory facilities and funds in a country facing many other health problems, this is not an option at present. The implementation of syndrome based treatment algorithms, adapted to the Nicaraguan context, should, however, be given serious consideration. Although in women such algorithms will miss a considerable proportion of cases while leading to unnecessary treatments in others,29 we have observed a substantial decline in STD rates among sex workers following the implementation of algorithms together with a health education and condom promotion programme.30

Unfortunately, official STD treatment guidelines do not follow a syndrome based approach.31 In Nicaragua, there is a threat of an epidemic of heterosexually transmitted human immunodeficiency virus (HIV) infections.32 In a large randomised community intervention trial in Tanzania, an intervention including syndromic STD management, continuous availability of drugs and health education has recently been shown to substantially reduce the incidence of HIV infections.33 In Nicaragua, levels of STD and HIV related knowledge and condom use are still low, and particularly low among young people and young women.34 Health education programmes in and out of school along with improved management in the management of patients with sexually transmitted diseases are urgently needed in Nicaragua.

We are grateful to all the women who participated in this study. We also thank all the medical staff of the clinics involved. Special thanks are due to Reyna Castro (León), Marc Isler (Bluefields), Giocconda Espinoza (Corinto), Karin Volken (Matagalpa) and others. This study was supported by the AIDS Task Force of the European Community, Medicus Mundi Switzerland, CARITAS Switzerland and Gruppe für Entwicklungszusammenarbeit (GBAR), Basle, Switzerland.


22 Reeves WC, Quiloz E. Prevalence of sexually transmitted diseases in high-risk women in the republic of Panama. Sex Transm Dis 1987;14:69–74.


27 Espinoza F, Egger M, Herrmann B, Iler M, Volken K, Davey Smith G. STD in Nicaragua: population rate esti-


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