Prevalence of Chlamydia trachomatis Infection Diagnosed by Polymerase Chain Reaction in Female Sex Workers in a Northern Mexican City

Luis Francisco Sánchez-Anguiano1, Nadia Velázquez-Hernández1, Fernando Martín Guerra-Infante2, Marisela Aguilar-Durán1, Alma Rosa Pérez-Álamos1, Sergio Estrada-Martínez1, José Antonio Navarrete-Flores1, Ada Agustina Sandoval-Carrillo1, Elizabeth Irasema Antuna-Salcido1, Jesús Hernández-Tinoco1 and Cosme Alvarado-Esquivel1*

1Institute for Scientific Research “Dr. Roberto Rivera-Damm”, Juárez University of Durango State, Avenida Universidad S/N, 34000 Durango, Mexico
2Faculty of Medicine and Nutrition, Juárez University of Durango State, Avenida Universidad S/N, 34000 Durango, Mexico

Keywords: Chlamydia trachomatis, prevalence, female sex workers, case-control study

Purpose: We aimed to determine the association between Chlamydia trachomatis infection and female sex work, and the association between sociodemographic, obstetric, and behavioral characteristics of female sex workers and C. trachomatis infection.

Methods: Through a case–control study design, we studied 201 female sex workers and 201 age-matched women without sex work in Durango City, Mexico. C. trachomatis DNA was detected in cervical swab samples using polymerase chain reaction.

Results: C. trachomatis DNA was detected in 32 (15.9%) of the 201 cases and in 6 (3.0%) of the 201 controls (odds ratio [OR] = 6.15; 95% confidence interval [CI]: 2.5–15.0; P < 0.001). The frequency of infection with C. trachomatis in female sex workers did not vary (P > 0.05) regardless of the history of pregnancies, deliveries, cesarean sections, or miscarriages. Regression analysis of the behavioral characteristics showed that infection with C. trachomatis was associated only with consumption of alcohol (OR = 2.39; 95% CI: 1.0–5.71; P = 0.04).

Conclusions: We conclude that C. trachomatis infection is associated with female sex work in Durango City, Mexico. This is the first age-matched case–control study on the prevalence of C. trachomatis infection in female sex workers in Mexico using detection of C. trachomatis DNA in cervical samples.

Keywords: Chlamydia trachomatis, prevalence, female sex workers, case-control study

Introduction

Chlamydia trachomatis (C. trachomatis) is a ubiquitous, obligate, intracellular Gram-negative bacterial pathogen [1]. Humans are the only natural host of C. trachomatis [2]. This bacterium remains a significant public health burden worldwide [3]. It is estimated that 100 million cases of C. trachomatis infection occur annually [4]. C. trachomatis is the pathogen that is most often transmitted by sexual contact [5]. The clinical spectrum of infection with C. trachomatis varies from asymptomatic to several acute or chronic, local, and systemic diseases such as trachoma, ocular-genital, and neonatal diseases [6–7]. Infections with C. trachomatis of the lower female genital tract are frequently asymptomatic, undiagnosed, and untreated [4]. Pelvic inflammatory disease attributed to ascending genital C. trachomatis infections can result in ectopic pregnancies and infertility in women [4–5]. In addition, a recent meta-analysis demonstrated that individuals infected with C. trachomatis have a higher risk of cervical cancer [8]. C. trachomatis causes inclusion conjunctivitis of the newborn, with the female birth canal being the reservoir [9]. In addition, infection with C. trachomatis causes pneumonia and sepsis [10]. In men, C. trachomatis causes urethritis and epididymitis [11].

Little is known about the epidemiology of C. trachomatis infection in Mexico. The prevalence of C. trachomatis in women in Mexico has been determined in several population groups; for instance, 4% prevalence was reported in women in Cuernavaca City [12]. 3.3% prevalence was found in women with leucorrhea in Mexico City [13], and 7.3% prevalence was reported in women in rural and suburban Oaxaca State [14]. However, female sex workers have shown higher prevalence of C. trachomatis infection. Uribe-Salas et al. [15] reported 11.1% prevalence of C. trachomatis in female sex workers in Mexico City. Whereas in 3 northern Mexican states, prevalence between 12.4% and 16.6% were found in female sex workers in Mexico City. Whereas in 3 northern Mexican states, prevalence between 12.4% and 16.6% were found in female sex workers [16–17]. In the present study, by using a different study design (case–control) and laboratory method (polymerase chain reaction [PCR]) compared to those used in previous studies in female sex workers in Mexico, we aimed to determine the following: (1) the association between C. trachomatis infection and female sex work and (2) the sociodemographic, obstetric, and behavioral characteristics of female sex workers associated with C. trachomatis infection.
Materials and Methods

Study Design and Women Studied. Through a case-control study design, we studied female sex workers (cases) and women with occupations other than sex work (controls) in Durango City from November 2014 to May 2016. Female sex workers were enrolled at the Sanitary Inspection Clinic of the Municipal Government in Durango City, Mexico. The inclusion criteria for enrollment of female sex workers were female sex workers (1) registered in the Sanitary Inspection Clinic, (2) worked in the sex industry for at least one year, and (3) aged ≥18 years old. Control subjects were women without sex work. Controls were sexually active women and enrolled at the Clinic for Family Planning in the Institute for Scientific Research. This public clinic is in Durango City, Mexico, and is part of Juarez University of Durango State.

Sociodemographic, Obstetric, and Behavioral Characteristics of Cases. We recorded the sociodemographic, obstetric, and behavioral data of cases with the aid of a questionnaire. Data about age, residence, birthplace, education, socioeconomic status, history of pregnancies, cesarean sections, miscarriages, and deliveries from all sex workers were obtained. Behavioral factors included duration (years) in the sex industry, area of work (urban, suburban, or rural), sex work in Mexican states other than Durango or abroad, contact with semen during vaginal intercourse, condom use, practice of oral or anal sex, injuries at sex work, and consumption of alcohol, tobacco, or drugs.

DNA Extraction and Amplification. Cervical swab samples were obtained from participants and stored in a home-made 2SP (sucrose-phosphate) medium at −70 °C until analysis. DNA was extracted from cervical samples by proteinase K digestion and phenol–chloroform extraction. C. trachomatis DNA was detected by PCR using primers of theomp A gen of C. trachomatis [18]. These primers generate the amplification of a 129-base pair fragment. The amplification mixture contained 1.5 mM MgCl2, 0.3 μM primers, 1 unit of Taq polymerase (Promega, Madison WI, EUA), 0.16 mM dNTPs, 2 ng DNA, and buffer in a 50-μL reaction volume. This amplification mixture was denatured at 95 °C for 10 min. Then, samples were amplified in 34 cycles. Each cycle consisted of the following: denaturation at 95 °C for 1 min, annealing at 61 °C for 2 min, and extension at 70 °C for 1 min. Samples underwent a final extension at 70 °C for 10 min. Amplification products were analyzed by electrophoresis in 2% agarose gel and stained with 0.1% ethidium bromide.

Statistical Analysis. Data were analyzed with the software SPSS version 15.0 (SPSS Inc. Chicago, Illinois). We calculated the odds ratio (OR) with 95% confidence interval (CI) to assess the association between C. trachomatis infection and sex work occupation. In addition, the association between C. trachomatis infection and the sociodemographic, obstetric, and behavioral characteristics of the female sex work were analyzed by the Pearson's chi-squared test and the Fisher exact test (when values were 5 or less). Variables of sex workers with a P value ≤0.20 obtained by bivariate analysis were further analyzed by multivariate analysis. We calculated the ORs and 95% CIs by regression analysis with the Enter method. Statistical significance was set at P value <0.05.

Ethics. The Ethics Committee of the Institute for Scientific Research of the Juarez University of Durango State, Mexico, approved this project. Participation in the study was voluntary. Written informed consent was obtained from all cases and controls before enrollment.

Results

Four hundred and two women were enrolled in this study. Half (n = 201) of them were cases (female sex workers), and 201 were controls (age-matched women). Mean ages in cases and women tested

### Table 1. Sociodemographic characteristics of female sex workers and prevalence of C. trachomatis infection

| Characteristic       | Women tested | Prevalence of C. trachomatis infection |
|----------------------|--------------|----------------------------------------|
|                      | No. | No. | %   | P value |
| Age groups (years)   |     |     |     |         |
| ≤20                  | 10  | 0   | 0.0 |         |
| 21–30                | 82  | 14  | 17.1| 0.56    |
| 31–40                | 64  | 11  | 17.2|         |
| ≥41                  | 45  | 7   | 15.6|         |
| Birth place          |     |     |     |         |
| Durango State        | 163 | 24  | 14.7|         |
| Other Mexican state  | 38  | 8   | 21.1| 0.33    |
| Residence place      |     |     |     |         |
| Durango State        | 200 | 32  | 16.0|         |
| Other Mexican state  | 1   | 0   | 0.0 | 1.00    |
| Education (years)    |     |     |     |         |
| No education         | 4   | 0   | 0.0 |         |
| 1 to 6              | 58  | 11  | 19.0| 0.38    |
| 7–12                | 129 | 21  | 16.3|         |
| >12                 | 10  | 0   | 0.0 |         |
| Socio-economic status|     |     |     |         |
| Low                  | 39  | 8   | 20.5|         |
| Medium               | 160 | 24  | 15.0| 0.4     |

### Table 2. Correlation between behavioral characteristics of female sex workers and C. trachomatis infection

| Characteristic             | Women tested | Prevalence of C. trachomatis infection |
|----------------------------|--------------|----------------------------------------|
|                            | No. | No. | %   | P value |
| Duration in the sex industry (years) |     |     |     |         |
| 1 to 5                     | 20  | 1   | 5.0 | 0.14    |
| 6 to 11                    | 47  | 11  | 23.4|         |
| ≥11                        | 134 | 20  | 14.9|         |
| Area of work               |     |     |     |         |
| Urban                      | 196 | 32  | 16.3| 0.74    |
| Suburban                   | 2   | 0   | 0.0 |         |
| Rural                      | 1   | 0   | 0.0 |         |
| Sex activity in Mexican states other than Durango |     |     |     |         |
| Yes                        | 86  | 13  | 15.1| 1.0     |
| No                         | 113 | 19  | 16.8|         |
| Condom use                 |     |     |     |         |
| Yes                        | 115 | 19  | 16.5| 0.77    |
| No                         | 80  | 12  | 15.0|         |
| Contact with semen during vaginal intercourse |     |     |     |         |
| Yes                        | 86  | 13  | 15.1| 0.74    |
| No                         | 113 | 19  | 16.8|         |
| Practice of oral sex       |     |     |     |         |
| Yes                        | 121 | 16  | 13.2| 0.18    |
| No                         | 79  | 16  | 20.3|         |
| Practice of anal sex       |     |     |     |         |
| Yes                        | 17  | 1   | 5.9 | 0.31    |
| No                         | 182 | 31  | 17.0|         |
| Injuries during sex activity |     |     |     |         |
| Yes                        | 12  | 2   | 16.7| 1.0     |
| No                         | 187 | 30  | 16.0|         |
| Alcohol consumption        |     |     |     |         |
| Yes                        | 36  | 10  | 27.8| 0.03    |
| No                         | 165 | 22  | 13.3|         |
| Tobacco consumption        |     |     |     |         |
| Yes                        | 41  | 5   | 12.2| 0.63    |
| No                         | 160 | 27  | 16.9|         |
| Drug use                   |     |     |     |         |
| Yes                        | 13  | 3   | 23.1| 0.43    |
| No                         | 188 | 29  | 15.4|         |
and controls were 33.06 ± 9.76 (range: 18–67) years old and 33.08 ± 9.79 (range: 17–67) years old, respectively (P = 0.98). \textit{C. trachomatis} DNA was detected in 32 (15.9%) of the 201 cases and in 6 (3.0%) of the 201 controls. The difference in the prevalence of \textit{C. trachomatis} infection found in cases and controls was statistically significant (OR = 6.15; 95% CI: 2.5–15.0; \textbf{P} < 0.001). Bivariate analysis of socioeconomic characteristics of female sex workers showed no association (\textbf{P} > 0.05) between \textit{C. trachomatis} infection and age, birthplace, residence, educational level, or socioeconomic status (Table 1). The frequency of infection with \textit{C. trachomatis} in female sex workers did not vary (\textbf{P} > 0.05) regardless of the history of pregnancies, deliveries, cesarean sections, or miscarriages. With respect to behavioral characteristics, the variables, namely, duration in the sex industry, practice of oral sex, and alcohol consumption, showed \textbf{P} values ≤0.20 by bivariate analysis (Table 2), whereas other behavioral characteristics including area of work, sex work in Mexican states other than Durango or abroad, contact with semen during vaginal intercourse, condom use, practice of anal sex, injuries at sex work, consumption of tobacco, or drugs showed \textbf{P} values >0.20 by bivariate analysis. Regression analysis of the 3 behavioral characteristics with \textbf{P} values ≤0.20 obtained by bivariate analysis showed that the infection with \textit{C. trachomatis} was associated only with consumption of alcohol (OR = 2.39; 95% CI: 1.0–5.71; \textbf{P} = 0.04) (Table 3).

**Discussion**

The epidemiology of \textit{C. trachomatis} infection in female sex workers in Mexico has been scantily studied. We therefore aimed to determine the association between \textit{C. trachomatis} infection and the occupation of female sex worker in Durango City, Mexico. We found a significantly higher frequency of \textit{C. trachomatis} DNA in cervical samples from female sex workers than in those from control women without sex work occupation. This finding indicates that \textit{C. trachomatis} infection is associated with the occupation of female sex worker. This age- and gender-matched case–control study thus demonstrates that female sex workers in Durango City have an increased risk of \textit{C. trachomatis} infection. Comparison of our results with those reported in studies of female sex workers in other countries using PCR methodology. The prevalence (15.9%) of \textit{C. trachomatis} infection found in our study is higher than the 6.6% prevalence of \textit{C. trachomatis} endocervical infection reported in female sex workers in Hungary using plasmid PCR [21]. In contrast, the prevalence found in our study is lower than a 28.5% prevalence of \textit{C. trachomatis} cervical infection found in female sex workers in Dakar, Senegal, using PCR [22]. The association between \textit{C. trachomatis} infection and female sex workers found in our study agrees with the same association found in a study in Bangladesh, where researchers found a significantly higher prevalence value of \textit{C. trachomatis} infection in 40 female sex workers (58%) than in 110 sexually active women (27%) using immunochromatography test and/or plasmid PCR [23].

We found an association between \textit{C. trachomatis} infection and alcohol consumption in female sex workers. In a search of this association in the biomedical literature, we did not find any report. It is possible that alcohol consumption in female sex workers might be linked to risky behavioral factors for \textit{C. trachomatis} infection. Further research about this association should be conducted.

**Conclusions**

We conclude that \textit{C. trachomatis} infection is associated with female sex work in Durango City, Mexico. This is the first age- and gender-matched case–control study on the prevalence of \textit{C. trachomatis} infection in female sex workers in Mexico using detection of \textit{C. trachomatis} DNA in cervical samples. The association between \textit{C. trachomatis} and consumption of alcohol deserves further investigation.

**Funding Source**

This study was financially supported by Juarez University of Durango State, Mexico.

**Authors’ Contributions**

LFSA and NVH conceived the study protocol, performed the data analysis, and wrote the manuscript. FMGI and JHT performed data analysis and wrote the manuscript. JANF and SEM obtained the samples and clinical data. MAD, ARPA, AASC, and EIAS performed laboratory tests and data analysis. All authors read and approved the final version of the manuscript.

**Conflict of Interest**

The authors declare that no conflict of interest exists.

**References**

1. Hooppaw AJ, Fisher DJ. A Coming of Age Story: \textit{Chlamydia} in the post-genetic era. Infect Immun 2015;84:612–21. doi: 10.1128/IAI.01186-15.
2. Witkin SS, Minis E, Athanasou A, Leizer J, Linhares IM. \textit{Chlamydia trachomatis}: the persistent pathogen. Clin Vaccine Immunol. 2017;24:e00203–17. doi: 10.1128/CVI.00203-17.
Chlamydia and Sex Workers

3. Nans A, Ford C, Hayward RD. Host-pathogen reorganisation during host cell entry by Chlamydia trachomatis. Microbes Infect. 2015;17:727–31. doi: 10.1016/j.micinf.2015.08.004.

4. Hafner LM. Pathogenesis of fallopian tube damage caused by Chlamydia trachomatis infections. Contraception. 2015;92:108–15. doi: 10.1016/j.contraception.2015.01.004.

5. Stock I, Henrichfreise B. Infections with Chlamydia trachomatis. Med Monatsch Pharm. 2012;35:209–22; quiz 223–4.

6. Budai I. Chlamydia trachomatis: milestones in clinical and microbiological diagnostics in the last hundred years: a review. Acta Microbiol Immunol Hung. 2007;54:5–22.

7. Karim S, Souho T, Bennani B. Cervical cancer induction enhancement potential of Chlamydia trachomatis: A systematic review. Curr Microbiol. 2018. doi: 10.1007/s00284-018-1439-7.

8. Zhu H, Shen Z, Luo H, Zhang W, Zhu X. Chlamydia trachomatis infection-associated risk of cervical cancer: A meta-analysis. Medicine (Baltimore). 2016;95:e3077. doi: 10.1097/MD.0000000000003077.

9. Darville T. Chlamydia trachomatis infections in neonates and young children. Semin Pediatr Infect Dis. 2005;16:235–44.

10. Lopez-Hurtado M, Guerra-Infante FM. Early Neonatal infection by Chlamydia trachomatis. J Infect Dis Ther. 2014;2:5. doi: 10.4172/2332-0877.1000158.

11. Acosta-Cázares B, Ruiz-Maya L, Escobedo de la Peña J. Prevalence and risk factors for Chlamydia trachomatis infection in low-income rural and suburban populations of Mexico. Sex Transm Dis. 1996;23:283–8.