Detection of *Chlamydia trachomatis* by immunological methods in adult and adolescent female population in Cuiabá, Mato Grosso

Detecção de chlamydia trachomatis através de testes imunológicos em população feminina adolescente e adulta na grande Cuiabá, Mato Grosso

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ABSTRACT

Introduction: Worldwide, *Chlamydia trachomatis* infection remains a major public health problem, especially for sexually active young adults. **Objective:** To investigate the sexually transmitted disease by *Chlamydia trachomatis* in adolescents and young women aged 15–25 years from Cuiabá and Várzea Grande, Mato Grosso, Brazil, through the ELISA and direct immunofluorescence methods. **Methods:** A cross-sectional quantitative study of endocervical samples from 328 nonpregnant, sexually active women who received care in basic health units. Endocervical samples were collected and *C. trachomatis* antigens detected by ELISA and direct immunofluorescence methods. **Results:** A total of 11 positive samples were obtained with ELISA (3.4%) and 69 with direct immunofluorescence (24.4%). The largest number of cases occurred in the 16–25 years age group (24.39%). **Conclusion:** The rate of positive cases observed was representative, similarly to those found in other studies, and, therefore, indicating Chlamydia strains circulating in the population studied. Amplification of prophylactic, diagnostic, and therapeutic measures in public health services will be an important step to counter the spread of sexually transmitted diseases, including genital infection by *C. trachomatis* in the female population.

Keywords: *Chlamydia trachomatis*; enzyme-linked immunosorbent assay; immunologic tests.

RESUMO

Introdução: Mundialmente, a infecção por *Chlamydia trachomatis* continua sendo um importante problema de saúde pública, especialmente para adultos jovens sexualmente ativos. **Objetivo:** Investigar doença sexualmente transmissível por *Chlamydia trachomatis* em adolescentes e jovens do sexo feminino, na faixa etária de 15 a 25 anos de idade em Cuiabá e Várzea Grande, Mato Grosso, através dos métodos imunológicos de ELISA e imunofluorescência direta. **Métodos:** Estudo de corte transversal quantitativo de amostras endocervicais de 328 mulheres sexualmente ativas, não grávidas, que frequentaram as Unidades Básicas de Saúde. Amostras endocervicais foram coletadas, sendo a detecção dos antígenos de *Chlamydia trachomatis* realizada pelos métodos ELISA e imunofluorescência direta. **Resultados:** Foram obtidas 11 amostras positivas por meio do ELISA (3,4%) e 69 pela imunofluorescência direta (24,4%). Observou-se elevado número de casos entre 16 a 25 anos (24,39%). **Conclusão:** O índice de casos positivos observado foi representativo, assemelhando-se aos encontrados em outros estudos e denotando, portanto, uma circulação de cepas de clamídia na população estudada. A amplificação das medidas profiláticas, diagnósticas e terapêuticas nos serviços públicos de saúde será um passo importante para conter o avanço da doença sexualmente transmissível, inclusive a infecção genital por *Chlamydia trachomatis* na população feminina.

Palavras-chave: *Chlamydia trachomatis*; ensaio de imunoadsorção enzimática; testes imunológicos.

INTRODUCTION

The WHO estimates that there are 105.7 million new cases of *Chlamydia* worldwide, with 3 to 4 million of these only in the United States of America (USA), with over 1.4 million cases reported in 2011[1-5], 5 million in Eastern Europe, and 34 million in subSaharan Africa and southeast Asia[5-9]. Worldwide, it occurs more frequently in sexually active young adults, generally aged younger than 20 years, and it is nearly three times higher in the 14–24 years age group[5,7,9,10]. It currently represents the leading cause of female infertility, but the most common infections are urethritis and cervicitis, which, if untreated, can lead to serious consequences in the reproductive tract such as ectopic pregnancy and infertility[6,8-10]. About 50% of infected men and 70 to 75% of infected women are asymptomatic. The infection is transmitted during sexual contact and to the newborn at birth and may cause neonatal conjunctivitis or pneumonia[5,6,8-10]. Over 100 million individuals of both sexes worldwide are infected with *Chlamydia* at some point in their lives[6,9,10]. Among male subjects, the prevalence is comparable with that of risk populations, ranging from 15 to 20%, while in the asymptomatic population, it is between 3 and 7%. In female subjects, the infection rates of asymptomatic populations are between 3 and 10%, while in high-risk populations they are above 20%[1]. Because of this high prevalence, the Centers for Disease and Control Prevention in the United States (CDC) have

Study conducted in MT Laboratório, Health Secretariat of the State of Mato Grosso – Cuiabá (MT), Brazil.

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recommended an annual screening for all sexually active women aged younger than 26 years[1,11].

In Brazil, epidemiological data about the infection are scarce, with variations between 6 and 20% depending on the methodology used for the diagnosis and the population[12-14]. However, according to the STD/AIDS coordination, in Brazil, there are about two million new cases annually[5,7,15]. Although this sexually transmitted disease (STD) often occurs asymptomatically, and despite its high incidence in the human population, it is not communicable in our country[1,7,15].

Cytological analysis for the detection of *Chlamydia trachomatis*, available since 1907, was the first method used in cell samples and inclusion conjunctivitis secretions in infants[1,6]. Other methods, such as complement fixation test, cell culture, and hybrid capture, were subsequently developed, and the last two show high sensitivity and specificity[16-19]. Nowadays, modern laboratory tests are available, using immunological and molecular techniques based on nucleic acid amplification, such as polymerase chain reaction (PCR)[16-19]. Thus, faced with a scenario of limited information on the prevalence of genital CT infection in our country, particularly, in the state of Mato Grosso, this study was conducted.

OBJECTIVE

To investigate the diseases sexually transmitted by *Chlamydia* among sexually active adolescents and young adults of reproductive age, using two methods: enzyme-linked immunosorbent assay (ELISA) and direct immunofluorescence (DIF).

METHODS

Between May 2009 and December 2011, a cross-sectional quantitative study was conducted with a random input of 328 endocervical samples of sexually active young women, aged 15–25 years, who received care in the basic health units (BHU) of Cuiabá (including 56 samples from the municipality of Varzea Grande), Mato Grosso. The sample size calculation was based on the arithmetic average obtained from several prevalence values in Brazil and other Latin American countries, USA, among others, estimated at 15%, obtained in recent articles.

The women who responded to the questionnaire were considered eligible to participate in the study and signed the informed consent (IC) approved by the Research Ethics Committee of Hospital Universitário Júlio Müller da Universidade Federal de Mato Grosso (UFMT) under protocol no. 463 12/03/2008 and of Universidade Federal de São Paulo (UNIFESP) under protocol no. 076208. The patients were approached during the course of their gynecologic cancer preventive examination, according to the individual profile. Antisepsis of the external and internal genitalia was performed, according to the Clinical Microbiology Guide for Infection Control in Health Care[20], e and the STD Control Manual[21] issued by the Ministry of Health. These procedures were performed by nurses and/or nursing technicians responsible for the performance of gynecological cancer preventive examinations, who have received specific training for *Chlamydia* collection, which consisted of inserting a speculum (without lubricant) in the vagina of non-menstruating women, without any other bleeding, with no use of vaginal douches and creams the day prior, with no use of antimicrobials, and at least three days of sexual abstinence. Then, with the aid of an Ayres clamp, with cotton on the tip, moistened with water, the excess mucus was collected from the vaginal opening and the endocervix, proceeding with the further insertion of the dacron swab in the endocervical canal until the tip was no longer visible, gently rotating for a few seconds, scraping the uterine cervix, removing it, and avoiding contact with the vaginal wall. A brush was also used, whose content was deposited in two tubes containing saline solution 0.85%, one of which was used for the ELISA test and the other stored in the Virology Section of MT-Laboratório at a temperature of -70°C for further testing by molecular methods. The endocervical content used in the CT search with ELISA was placed in the refrigerator in health facilities, not exceeding a period of 48 hours, before being transported to the laboratory and stored in saline solution 0.85%. Processing of the samples was performed according to the manufacturer's instructions as described later.

The transportation of clinical specimens was performed according to the usual precautions in a Styrofoam box filled with ice and stored in a refrigerator for 48 hours until being sent to the laboratory. Upon receipt in the laboratory, the samples were placed between 2 and 8°C up to the moment of analysis, within 7 days at the most. The detection methodology employed by ELISA used the miniVIDAS® equipment, a multiparameter automated system for immunoassay that uses the enzyme-linked fluorescence assay (ELFA) technology, combining the ELISA methodology with the final fluorescence reading, which is composed of a multiparameter analytical module, a computer, and a printer.

VIDAS *Chlamydia* CHL is an automated qualitative test on the VIDAS system, which allows the detection of the fixed lipopolysaccharide antigen (LPS) *Chlamydia* from endocervical and urethral specimens using the ELFA technique through the use of specific marked monoclonal antibodies (more than ten known *Chlamydia* antigens are detected). The test exhibits a sensitivity of around 70–100% and specificity of 95%. The beginning of the assay associated an enzyme immunoassay to a final fluorescence detection (ELFA), in which the sample is subjected to suction and dispensation cycles during a given time.

For the DIF test, the endocervical samples were collected as described earlier, using another swab and conducting a smear or imprint on slides for *Chlamydia* DIF and fixed with ethanol. After the complete evaporation of the fixative, the slides were wrapped in aluminum paper and identified. All the samples from each patient, the test tube and the slides were placed in plastic bags and containers and, after identification, stored in a refrigerator until the moment they were sent to the laboratory, for the maximum period of 24 hours. These slides were subjected to the technique using a Pathfinder *C. trachomatis* Direct Specimen kit (BIO-RAD, USA), according to the manufacturer's recommendations and examined with a fluorescence microscope.

The smears were investigated for the presence of fluorescent green elementary bodies (EB). The samples were considered positive if they presented at least five inclusion bodies per slide, cut-off recommended by the manufacturer or, if fewer, when there was no doubt they were inclusion bodies rather than artifact. The absence of *Chlamydia* bodies was considered negative. After the coloration, the slides were examined under fluorescence binocular microscope (Axioscope-A1, CARL ZEISS).

Upon completion of the ELISA and DIF laboratory tests, all the patients with positive results were referred to specialists for medical treatment.

Pearson's χ^2-test, Fisher's exact test, and Student's *t*-test were used for the correlation of variables.

RESULTS

All the specimens collected were tested with the DIF and ELISA methods, with a total of 80 positive cases, of which 11 were detected by ELISA (3.4%) and 69 by the DIF method (24.4%) and 10 positive samples by ELISA were confirmed by DIF. Subsequently, a PCR test was validated using primers to amplify the gene of the major outer membrane protein (MOMP) in the Microbiology Laboratory of Universidade Federal de Mato Grosso, with 50 random samples, of which 15 were positive for CT and showed a positive correlation with the analyzed tests. The positivity rates for the different regions of the Metropolitan region of Cuiabá remained highly homogeneous, except in the western region, where a low percentage of occurrences in the population was observed. The highest number of positive cases (24.39%) occurred in the 15–25 years age group **(Table 1)**.

Variables such as drug use (3.75% of the samples), alcohol consumption (6.25% of the samples), and smoking (7.5% of the samples) and the occurrence of induced abortion (10% of positive cases) were associated with the risk of infection by CT but showed no significant difference for the population **(Table 2)** considering the significance level of 5%.

For other risk factors studied, such as risky sexual behavior, illicit drug use, use of protective barriers, age, socioeconomic status, among others, there was no association with STD positivity for CT.

As for the stratification of income, a significant portion of the positive population was poor or was informally employed **(Table 3)**.

About 50% of the positive cases were found among those who reported living together in formal or informal marriage. With regard to ethnicity, the largest portion of the study population was white, but the greatest number of positive cases was observed in the brown ethnicity (42.5%).

Regarding the number of sexual partners, approximately, 27.4% of patients reported that they possessed more than two sexual partners in the previous 12 months. The largest number of cases, however, was in the group of those with steady partner.

Table 1 – Positivity for *Chlamydia trachomatis* according to age in 328 women assisted from 2009 to 2011 in basic health units in Cuiabá, Mato Grosso.

Age range (years)	n	%	Positive	Intragroup rate (%)	Positive rate (%)
15–16	26	7.9	6	23.0	7.5
17–18	46	14	13	28.3	16.3
19–20	73	22.3	18	24.6	22.5
21–22	62	18.9	20	32.0	25.0
23–24	67	20.4	11	16.4	13.7
25	54	16.5	12	22.2	15.0
Total	328	100	80	–	100

DISCUSSION

CT is the causative pathogen of different clinical infections in humans, especially urogenital which in general are asymptomatic and can occur more frequently in subjects with high-risk sexual behavior. The severe cases are more common in women, especially in adolescents and young people up to 20 years of age [2,10]. The prevalence rates found in Brazil are variable and found by different methods[5,17,18]. According to the reports by the Health Surveillance Secretariat, STD and AIDS Program, the overall prevalence of CT infection, in 2005, was 9.2% for both sexes and 7.3% only in women[15]. The overall rates for each of the cities participating in the study, in descending order, were: Rio de Janeiro (15%), Porto Alegre (12.2%), Vitória (10.7%), São Paulo (9.1%), Manaus (7.8%), Goiânia (7.6% in the female population and 5% in asymptomatic males), and Fortaleza (4.7% for both sexes)[15,22-26]. More recent data have established a prevalence of 56.45% in the endocervical samples from 287 women in São Paulo and Santa Catarina by the PCR method[27]. In another study, conducted in Manaus, with samples from 100 pregnant women by the same method demonstrated positivity of 11%[28]. In this study, there was a high number of positive cases (24.39%) of CT in young people aged 15–25 years. This prevalence was higher than that found by Araújo in the city of Goiania, in adolescents and young women, estimated at 19.6% and lower than those found in São Paulo and Santa Catarina[27,28]. Statistical analyses of this trial demonstrated an association of the variable age with the risk of *Chlamydia* infection (p=0.0060). Therefore, we consider that the prevalence of this STD in all groups was high and, according to numerous scientific publications in this regard, our data reinforce that age is a risk factor for genital CT infection and low socioeconomic conditions.

In the Brazilian midwest, there is little epidemiological data and the number of reported cases is below estimates, suggesting

Table 2 – Positivity for *Chlamydia trachomatis* infection according to the risk factors observed in adolescents and young people assisted from May 2009 to December 2011 in basic health units in Cuiabá, Mato Grosso.

Risk factors	n	%	Positive	Intragroup rate (%)	Positive rate (%)
Drug user	8	2.4	3	37.5	3.7
Alcohol user	21	6.4	5	23.8	6.2
Smoker	19	5.8	6	31.6	7.5
Abortion history	40	12.2	8	20	10
Total	88	26.8	22	112.9	27.4

Table 3 – Positivity for genital *Chlamydia trachomatis* infection in relation to the family income of adolescents and young women served from 2009 to 2011 the basic health units in Cuiabá, Mato Grosso.

Income (R$)	n	%	Positive	Intragroup rate (%)	Positive rate (%)
Up to 700	86	26.2	24	27.9	30.0
Between 700 and 4,000	87	26.5	14	16.0	17.5
Noninformed/no income	155	47.3	42	27.1	53.7
Total	328	100.0	80	71.0	100.0

underreporting of cases. Perhaps, this is owing to the prevalence of self-medication, the lack of specialized diagnostic laboratories, misinformation, or even the lack of a better welfare policy to this STD. In Mato Grosso do Sul, a rate of 6.64% was found in a group of pregnant women using the enzyme immunoassay method[29]. In some groups with high-risk behaviors, the prevalence rates may typically vary between 20 and 30%[15]. Other STDs have been detected at a high frequency in the state of Mato Grosso, such as HIV, syphilis, human papillomavirus (HPV), and gonorrhea, among others. The increased incidence of STDs is, probably, related to a marked migration of young individuals from around the country, particularly, because the state of Mato Grosso, in recent years, has become an attractive region to those searching for better opportunities.

Regarding the use of protective measures and/or contraceptives, the highest percentage of positive cases (75%) corresponded to those who reported using a condom or the pill. Among the supporters of one of the methods, 12.5% of positive cases occurred among those who adopted oral contraceptives, and 7.5% occurred among those who used condoms as a protective barrier. With regard to the number of sexual partners and marital status, there was a higher positivity among patients who reported having a single partner and among those who said they adhered to the protective measures. The lack of association in this case may be related to the fact that women with a regular partner feel safer and do not use condoms. Although the occurrence of STDs is generally associated with sexual promiscuity, currently, there is a larger risk in monogamous individuals owing to occasional contamination of the partner in extramarital relationships. The association observed is related to the southern region of the metropolitan region of Cuiabá, where the highest number of positive cases (54%; $p<0.05$) was found. This region was situated on the outskirts of the capital, where there is a high population density, high demand for care in health centers, and a large number of individuals living in poor socioeconomic conditions.

Among the ethnic group, there was a higher number of positive cases in the mulatto group, perhaps owing to the prevalence of this phenotype in the population of Mato Grosso. However, there were no significant differences between white or black skin groups, although some authors consider the existence of differences in the prevalence of *Chlamydia* infection among ethnic groups[30,31].

Various methods are employed for the diagnosis of *Chlamydia*. Although the cell culture is considered as the gold standard for *Chlamydia* detection, with a specificity of 100%, it is a low sensitivity technique (about 50 to 80%) and complex, costly, difficult to perform, time consuming, and depends on good infrastructure but with a low probability of contamination and the advantage of allowing the performance of antimicrobial susceptibility tests, antigenic characterization, and genotyping[5,13,16-19]. On the other hand, immunological techniques are useful for screening because of their simplicity of execution, good reproducibility, and efficiency and owing to often showing a high sensitivity and specificity[16,17]. Through immunoassays, antigens such as LPS and MOMP can be detected. However, in these tests, the sensitivity, specificity, and predictive values are highly variable, being less sensitive than culture and DIF[3,13,16,19]. The advantage of these techniques takes place in specific cases, because it allows the screening of large numbers of samples, and it is also more constantly suggested for epidemiological studies and diagnoses of systemic infections[17]. In less developed regions, the use of the DIF technique is recommended, because the cold storage during transport is not necessary, and it can be applied to samples from the conjunctiva, urethra, and rectum and endocervical samples. Through this method, the EB are observed directly owing to the specific fluorescein-labeled antibody-antigen reaction[5,16,17,22]. It is a quick technique, in which only 30 minutes are sufficient to diagnose the urogenital infection, thus constituting a useful tool in diagnostic laboratories, where the cell culture and more modern and sensitive methods are not available[6,12,16,17]. As a disadvantage, the DIF exhibits the need for skilled microscopist and expensive fluorescence equipment. Intra- and interspecific cross-reactivity also occur with LPS of Gram-negative bacteria, while false-positive results are rarely observed with the use of the MOMP epitope (species-specific)[5,6,13,16,17]. The combination of two techniques, such as cell culture and immunofluorescence, was recommended as the gold standard, expanded until the middle of 1990. However, the diagnosis by PCR has replaced other techniques for its speed, more reliable reproducibility and, currently, low cost. The methods based on the amplification of nucleic acids have demonstrated a high positive predictive value, presenting the advantage of being usable with urethral, cervical, vaginal, and urine specimens[30,31].

Several factors can interfere with the determination of the prevalence of this STD, such as the laboratory resources available, ecology of the bacteria, the sexual behavior of population groups, therapeutic interference, among others. Thereby, prior studies of the population are advisable, particularly, for adolescents, combined with the choice of sensitive and specific detection methods and individualized analysis of each case[2]. Possible biases regarding our results showing a large discrepancy between the results of ELISA and DIF may be owing to several factors that are difficult to be measured, but some hypotheses can be raised. For example, the false-positive and false-negative results may occur owing to bacterial urinary tract infections, cervical mucus contamination or vaginal secretions, nonspecific antigen–antibody reactions, inappropriate collection, and transport of samples[6,16,17]. A study using the ELISA, DIF, and PCR methods in 100 urethral and endocervical samples of male and female populations under high and medium risk of *Chlamydia* infection detected positivity rates of 3, 11, and 9%, respectively[32]. Of the positive samples for DIF, 72.73% were confirmed by PCR. Further studies regarding the performance of the ELISA test were carried out in different parts of India and the world, reproducing the same results[32]. Previous studies conducted by different authors with different techniques and distinct populations obtained mixed results regarding the observed prevalence. Using the DIF test on the population of both sexes, the lowest prevalence observed was 4.4% in a study conducted in 1987, and the highest, 23.1% in the female population[33]. Using the enzyme immunoassay, the samples were analyzed for endocervical and urethral secretions, obtaining a prevalence that ranged from 1.4 to 32%, both in the male population, in a comparative study conducted in 1992 by the same author. Therefore, we can conclude that enzyme immunoassays can provide low detection rates of *Chlamydia* antigens in symptomatic patients with reduced numbers of microorganism in the secretions, often as a result of previous antimicrobial therapy[32].

CONCLUSION

The rate of positive cases observed was representative, resembling the data found in other studies and denoting, therefore, *Chlamydia* strains circulating in the population studied, which deserves more attention for the control of its spread. The conduction of prophylactic, diagnostic, and therapeutic measures in public health services will be an important step to counter the spread of STDs, including genital CT infection in the female population.

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Conflicts of interests

The authors report no conflict of interests.

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