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High prevalence of *Chlamydia trachomatis* in pregnant women attended at Primary Health Care services in Amazon, Brazil

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ABSTRACT

Chlamydia trachomatis (CT) infection is often silent and can lead to long-term reproductive complications in women. In this study, we determined the prevalence of CT infection and possible associations between the presence of the infection and clinical-epidemiological variables in pregnant women attended at the Basic Health Units of the Coari city, Amazonas, Brazil. From July 2016 to March 2017, 164 pregnant women undergoing prenatal care were recruited. One hundred of these women were tested for CT infection using two types of samples: cervico-vaginal and urine. The diagnosis was confirmed by PCR with primers specific for the omp1 gene of CT chromosomal DNA. Of the 100 pregnant women, 18 (18%) had CT infection, 8 (8%) of which were positive in both samples, 7 (7%) only in the urine sample and 3 (3%) only in cervical-vaginal sample. There was moderate agreement (Kappa=0.55) and no statistically significant difference between sample types (p = 0.400). The mean age of infected women was 21.1 years (SD = 4.6). Of the clinical-epidemiological variables analyzed, "more than 2 partners in the last 12 months" (p = 0.022) and gynecological complaint of "pain after intercourse" (p = 0.020) were associated with CT infection. This study showed a high prevalence (18%) of CT infection among pregnant women in Coari / Amazonas. Urine sampales were as good as cervical-vaginal ones for the screening of CT infection during the prenatal period.

KEYWORDS: *Chlamydia trachomatis.* Pregnancy. Diagnosis. Amazonas.

INTRODUCTION

Pregnancy is a time of many changes in a woman's life, including hormonal and physiological changes and reduced immune activity, making her vulnerable to infections. Women, therefore, are more likely to acquire Sexually Transmitted Infections (STI) during pregnancy[1]. Among sexually transmitted pathogens, *Chlamydia trachomatis* (CT) infection is one of the most common, being associated with problems in women's reproductive health. Fifty to 80% of women infected with CT do not develop symptoms; these women are considered silent reservoirs of the pathogen and continue to transmit it sexually. The untreated infection allows CT propagation to the upper genital tract, which can lead to pelvic inflammatory disease (PID), ectopic pregnancy and infertility. During pregnancy, CT infection can lead to rupture of membranes, premature labor, miscarriage, low birth weight, conjunctivitis and neonatal pneumonia, among other complications[2-4].

Brazilian epidemiological studies show that CT infection rates in pregnant women range from 6.9% to 18%[5-7], and studies specifically conducted in the Amazon show a prevalence of CT infection in pregnant women of about 11%[8,9]. Countries

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such as Germany, Bulgaria, Bahamas, Canada, Estonia, Japan, Romania and Sweden are screening for prenatal CT infection in order to avoid pregnancy complications[10,11]. Other countries, such as Australia, the United States, New Zealand and Latvia, restrict prenatal screening of pregnant women aged ≤ 25 years and the ones at high risk[12]. In Brazil, public and private services do not routinely screen CT infection, even in pregnant women[1].

Urban areas of the Brazilian Amazon inland cities have no studies on CT infection rates in pregnant women. These urban areas located within the extensive territory of the Brazilian Amazon have a very precarious health system, and besides that, there are many isolated small villages, where access is possible only by river or little seaplanes, making it difficult for pregnant women to access adequate health services. Spontaneous abortions are frequent, and neonatal deaths are common due to the lack of human resources and adequate health care infrastructure for premature newborns. Also due to the great extent of the Amazonian territory, premature newborns depend on air transportation to the capital (Manaus) in order to receive intensive neonatal care, further increasing the difficulties and costs of the process. Therefore, knowing the epidemiology of CT infection in this population and seeking measures that may help reducing these complications are necessary. In this study, we determined the prevalence of CT infection in cervical and urine specimens of pregnant women attending prenatal care at the Basic Health Units of the city of Coari / Amazon.

MATERIALS AND METHODS

Study setting and population

Coari city, with a territorial area of 57,970,768 km^2, is in the region of the medium Solimoes (Solimoes River), 360 km away from the capital of Amazonas (Manaus). Its current estimated population is 84,000 inhabitants[13]. The estimated minimum number of pregnant women for this study was 58, based on the number of 1,729 births in the year 2013[14], and an expected prevalence of STI of 1.4% (according to data from DATASUS of STI infection in pregnant women of Coari in the year 2013), with a margin of error of 3% and 95% confidence interval. Basic Health Units (BHU) are places in which pregnant women perform prenatal care and there is infrastructure for the collection of vaginal samples. There are 12 BHU in Coari city that attend the urban population, and all of them were visited between July 2016 and March 2017. The inclusion criteria were: pregnant women in the second trimester of pregnancy (14 to 27 weeks), in order to minimize the risk of any association between collection and adverse events such as bleeding disorders, miscarriage, preterm delivery, premature rupture of membranes, among others, without maternal age limitation. Exclusion criteria were: any clinical situation of risk for gestation, such as vaginal bleeding, history of accidental fall, placenta previa, loss of amniotic fluid, dilation of the cervix, among others.

The total number of pregnant women registered was 164, of which 100 participated in the study. Of the 64 pregnant women who did not participate, 52 accepted the invitation made during visits to their homes but did not attend the scheduled sample collection, even after t the consultation was rescheduled. Twelve women were not able to participate because they presented some clinical condition of risk for the samples collection (according to exclusion criteria).

Study type and specimens

This is a descriptive and cross-sectional study, approved by the Research Ethics Committee of the Federal University of Amazonas (CAAE 50907415.3.0000.5020). The 164 registered pregnant women were visited in their homes. Those who accepted to participate and met the inclusion criteria were included after signing the consent form and answering a clinical-epidemiological questionnaire, totaling 100 women. On the days scheduled by BHU's professionals, the first urine of the day (20mL) from pregnant women was collected in a sterile cup. A cervical-vaginal sample was also collected (endocervix, ectocervix and vaginal wall) with the aid of a vaginal speculum and a swab. The swab was immersed in TRIS-EDTA (TRIS-HCl 10 mM and EDTA 1mM pH 8,0) buffer and the samples were transported in a thermal box (2 °C to 8 °C) and stored in the laboratory at -20 °C until processed.

Processing of samples

In a 15 mL falcon tube 14 mL of urine sample were centrifuged at 2,000 g for 20 min. The supernatant was discarded and the pellet was washed with PBS (Phosphate - Buffered Saline). The cervical-vaginal samples were vortexed and then 1 mL was transferred to a microtube. DNA extraction of urine and cervical-vaginal samples were performed using the commercial QIAamp Viral RNA Mini Kit (QIAGEN, Germany), according to the manufacturer's recommendations.

CT detection

CT detection was performed by PCR using the primer pair CT05/CT06 (5'-GAT AGCCAGCACAAAGAGAGCTAA

-3'/5'- TTTGTTTTCGACCGTGTTTTG CAAACA GATGTGAA -3'), which amplify a 280 bp region of the MOMP gene located within chromosomal DNA[15]. As the internal control of reactions the primers PCO3+/PCO4+ were used (5'-CTTCTGACACAACTGTGTTCACTA GC-3'/5' TCACCGCAACTTCATCCACGTTCACC-3'), which amplify a 110 bp fragment of the human β-globin gene[16] were used. The final volume of the reaction was 25 µL, containing 5 U of Platinum Taq DNA polymerase High Fidelity (Invitrogen, USA), 5 pmol of each primer, 2.5 µL of 10X reaction buffer, 50 mM $MgSO_4$, 10 mM dNTP, 5.0 µL of sample DNA and sterile water. Amplification was performed in a thermal cycler, under the following cycling conditions: initial denaturation of 94 °C for 30s, followed by 40 cycles of 94 °C for 30s, 58 °C for 1 min 30s, 68 °C for 2 min and a final extension of 68 °C for 5 min. In each reaction, a previously known CT positive control and a negative control with water instead of DNA were used. The amplified products were visualized on 1.5% agarose gel stained with ethidium bromide (1 µg / µL).

Statistical analysis

Data were tabulated in an electronic file created in the free Epi-Info program (v.7.2.1.0) (Center of Disease Control and Prevention – CDC)[17]. Statistical analysis was performed in the BioEstat program (v.5.0)[18]. Associations of CT infection and sociodemographic as well as behavioral characteristics were assessed using the chi-square test or the Fisher's Exact test. Statistical significance was set at $p < 0.05$. To evaluate the agreement between the two types of samples, the Kappa coefficient was used. Excellent agreement was defined as Kappa > 0.75[19].

RESULTS

The human β-globin gene fragment was amplified by PCR in all 100 DNA samples from pregnant women who participated in this study, both in urine and in cervical-vaginal samples, confirming the inexistence of amplification inhibitors in the DNA samples. The prevalence rate of CT infection was 18% (18/100). The prevalence of CT in urine samples was 15% (95% CI: 8.65 - 23.53) and in the cervical-vaginal samples was 11% (95% CI: 5.62 - 18.83). No significant difference was found between the two sample types (p= 0.400) (Table 1) and the Kappa coefficient calculated to evaluate the agreement among the samples was 0.55, showing a moderate agreement.

The patients' ages ranged from 13 to 41 years; the mean age was 22.9 years (SD 6.24). The majority of participants had low educational level (less than nine years of study), were married, reported two or more sexual partners in life, with the average age of the first intercourse of 15.2 years (SD = 2.19). The sociodemographic, behavioral and clinical characteristics of the studied population are in Table 2. These characteristics were analyzed in relation to CT infection and a statistically significant association of this infection was found with pregnant women who had had two or more sexual partners in the last 12 months (p=0,022) and who had a gynecological complaint of "pain after intercourse" (0,020) (Table 3).

Table 1 - Frequency of CT infection positivity in biological samples from 100 pregnant women recruited in the Basic Health Units of Coari city, Amazon, Brazil.

Sample	PCR		Positivity (IC95%)	p-Value*
	+	-		
Cervical	11	89	11% (5.62 – 18.83)	0.400*
Urine	15	85	15% (8.65 – 23.53)	

*Chi-square test

DISCUSSION

Chlamydia trachomatis (CT) infection is almost always asymptomatic so that infected people usually do not know their infection status and do not seek treatment. This chronic infection can cause long-term complications, including some related to the gestational period. CT-infected pregnant women may undergo preterm labor, premature rupture of membranes or give birth to low birth weight newborns. Infants born to mothers with CT may be infected during labor through the vaginal canal and thereby develop conjunctivitis and/or pneumonia[3].

This is the first study to detect CT in pregnant women in Coari city. In this town, a previous study was carried out with 361 sexually active women over 18 years of age who sought the BHU for routine gynecological appointment, and a prevalence of 6.4% of STI was found, a prevalence lower than those found in other Brazilian cities with similar population samples[20]. In our study with pregnant women, when we consider as positive any of the two type of sample (urine or cervical-vaginal), the prevalence of CT infection was 18%. In urine samples the prevalence of CT infection was 15%, which is among the highest rates found in pregnant women in studies conducted both in Brazil and in other countries. In Brazilian studies, we found rates of CT infection in urine samples from pregnant women ranging from 6.9% to 18%[5,6] and in other countries a prevalence between 6.4 and 18.1%[21-23]. In the cervical-vaginal samples, the prevalence of CT infection was 11% in our study, similar to the results found in other

Table 2 - Distribution of pregnant women recruited at the Basic Health Units of Coari city, Amazon, Brazil, according to socio-demographic, behavioural and clinical characteristics.

Variables (n=100)	n	%
Age (years)		
Mean ± SD	22.87 ± 6.24	
Amplitude	13-41	
Marital status		
Single	22	22.0
Married/Stable union	78	78.0
Age at first sexual intercourse (years)		
Mean ± SD	15.17 ± 2.19	
Amplitude	9-23	
Education (years)		
< 9 (primary/intermediate)	52	52.0
≥ 9 (secondary/higher)	48	48.0
Family income (minimum wages)[a]		
≤1	54	54.0
2	36	36.0
≥3	10	10.0
Occupation		
Housewife	50	50.0
Student	25	25.0
Others	25	25.0
Number of sexual partners over a lifetime		
1	20	20.0
≥ 2	80	80.0
Numbers of sex partners in last 12 months		
1	77	77.0
≥ 2	23	23.0
Number of pregnancies		
Primitive	48	48.0
>1gestation	52	52.0
Abortion story (n=52)		
Yes	12	12.0
No	40	40.0
History of preterm birth (n=52)		
Yes	08	8.0
No	44	44.0
Clinical complaints (n=69)[b]		
Pruritus	15	21.7
Vaginal discharge	45	65.2
Pelvic pain	35	50.7
Difficulty urinating	06	8.7
Pain after intercourse	02	3.0

[a] Minimum wage at the time of the study was 880.00 approximately 286 dollars; [b] It does not add up because more than one clinical complaint may occur.

studies conducted in Brazil or abroad using this type of sample[7-9,24,25].

These two types of sample appear to be equivalent for the diagnosis of CT by PCR. In our study, a moderate concordance was found between the samples (kappa = 0.55), and no statistically significant difference was found among these results (p = 0.400). In the study by Fang *et al*.[26], using urine samples, endocervical swab and vaginal swab collected by the participants, the authors found an excellent concordance between results (Kappa = 0.89). Regarding sensitivity and specificity, other studies have shown that urine and vaginal samples are equivalent for the diagnosis of CT and they emphasized the importance of using urine for the routine screening of CT infection[27,28]. In this way, urine samples are an excellent choice for the diagnosis of CT in pregnant women, since it is self-sampled, noninvasive and is already routinely used for detection of other infections during prenatal care[29,30].

In our study, we believe that the high rate of non-attendance of pregnant women (52 women) may be related to the discomfort and anxiety that cervical-vaginal collections always cause in women. If in our study we had used only urine, perhaps the refusal rate would not have been so high.

Both, Hybrid Capture (HC) method and PCR have been used for diagnosis of CT infection. In Brazil, the HC test has already been approved by the National Agency of Sanitary Surveillance (*Agência Nacional de Vigilância Sanitária* - ANVISA), and the Unified Health System (*Sistema Único de Saúde* - SUS) and included in the list of paid procedures. However, studies have shown that the sensitivity of the HC test ranges from 66.7% to 72.3% when compared to PCR, being inappropriate for CT screening due to this moderate sensitivity[31].

The PCR technique allows the amplification of DNA from minimal amounts of the CT DNA with high sensitivity and specificity, although with higher cost. Primers used in PCR for detection of CT may target plasmid DNA, omp1 gene in chromosomal DNA and 16S ribosomal gene. Many studies have used primers for plasmid DNA since there are 7 to 10 copies of it per bacterium, making the technique more sensitive. However, a study by Farencena *et al*.[32] described the isolation of a CT devoid of plasmid and Ripa and Nilsson[33], in Sweden, found a strain that had a 377 bp deletion in plasmid DNA between nucleotides 608 and 985 (known as the "Swedish variant"). In our study, to reduce the likelihood of false negative results , we used a primer pair for the omp1 gene, which is in chromosomal DNA coding for the main protein of the outer membrane of the bacterium, within a conserved region in all CT serotypes[15].

Table 3 - Sociodemographic, behavioral and clinical characteristics of pregnant women recruited at the Basic Health Units of Coari city, Amazon, Brazil, in relation to CT infection.

Variables (n=100)	*Chlamydia* identification				Total	*P*
	Negative (n=82)		Positive (n=18)			
	n	%	n	%		
Age (years)						0,226[c]
Mean ± SD	23.08 ± 6.36		21.15 ± 4.65		22.87 ± 6.24	
Marital status						0.165[b]
Single	16	72.7	06	27.3	22	
Married/Stable union	66	84.6	12	15.4	78	
Age at first sexual intercourse (years)						0.190[c]
Mean ± SD	15.30 ± 2.33		14.55 ± 1.29		15.17 ± 2.19	
Education (years)						0.392[a]
< 9 (primary/intermediate)	41	50.0	11	61.11	52	
≥ 9 (secondary/higher)	41	50.0	07	38.9	48	
Number of sexual partners over a lifetime						0.491[b]
1	17	85.0	03	15.0	20	
≥ 2	65	81.2	15	18.8	80	
Numbers of sex partners in last 12 months						0.022[b]
1	67	87.0	10	13.0	80	
≥ 2	15	65.2	08	34.8	20	
Number of pregnancies						0.851[a]
Primitive	39	81.2	09	18.8	48	
>1gestation	43	82.7	09	17.3	52	
Abortion story (n=52)						0.110[b]
Yes	08	66.7	04	33.3	12	
No	35	87.5	05	12.5	88	
History of preterm birth (n=52)						0.578[b]
Yes	07	87.5	01	12.5	8	
No	36	81.8	08	18.2	44	
Clinical complaints (n=69)						
Pruritus	13	86.7	02	13.3	15	0.11[d]
Vaginal discharge	35	77.8	10	22.2	45	0.55[d]
Pelvic pain	30	85.7	05	14.3	35	0.27[d]
Difficulty urinating	06	100	00	00	06	1.00[d]
Pain after intercourse	01	50.0	01	50.0	02	0.05[d]

N= Number of patients; Sd= standard deviation; [a]Chi-square test; [b] Ficher's exact test; [c] student test;[d] binominal test

Regarding the epidemiological profile of infected women, our results shave shsown that the mean age of infected pregnant women was lower than the uninfected ones (21.15 ± 4.65 e 23.08 ± 6.36, respectively), but with no statistically significant difference between groups (p=0,226). Studies have shown that CT infection was found predominantly in pregnant women up to 25 years old[5,7,21,34,35], showing that young women are at higher risk of acquiring this infection, which was also observed in this study. Studies have shown that the highest rates of CT infection are found in developing countries, among poorer and least educated[5,6,36] people, and this finding is in agreement with our results: our tested group is composed of women living in a poor and geographically isolated urban center, an inland town in the State of Amazonas, located in the Northern region of Brazil. Brazil is a country

of continental dimensions, showing great discrepancy in socioeconomical indexes between the South and the North of the country; and excepting for State capitals, most people living in cities of the Northern Region of Brazil have low levels of education and more difficult access to basic health services[37]. Most women in our study had monthly family income below R$ 880 (equivalent to about $286) and 52%reported having studied less than 9 years.

Some studies have shown that the prevalence of infection tends to be higher in single women and in those with different partners[5,20,34,35]. In our study, the prevalence of infection was higher in single women (27.3%) than in women living with fixed partners (15.4%), although differences were not statistically significant (p=0.165). The main risk factor for CT infection in this study was the highest number of partners in the last 12 months, with a prevalence of 44.4% among women who reported this condition. In this population, considering the history of preterm birth and spontaneous abortion - clinical outcomes related to CT infection - our study found no association between CT infection and these variables, which is in agreement with some Brazilian studies that also fail to find these associations[6,7]. On the contrary, other authors clearly found association between these variables and CT infection[4,5]. Although no association of CT infection with such of the outcomes was found, we consider that CT infections in these women may be recent and may still cause undesirable outcomes in future pregnancies.

CONCLUSIONS

In Brazil, during prenatal care, the routine screening of HIV, syphilis, hepatitis B and C, toxoplasmosis and rubella among pregnant women is recommended by the Brazilian Ministry of Health and it is performed. The high prevalence (18%) found in our study helps to increase the visibility of the problem of CT infection during pregnancy and reinforces the importance of including CT screening in prenatal care routine in Brazil. Screening for CT infection during prenatal care using urine samples and PCR (or even HC) as the diagnostic method is an opportunity to detect and treat women, to reduce the harm to the mother and child, as well as expenses with the undesirable outcomes. We consider that there is a need for cost effectiveness studies the inclusion of CT screening in prenatal care in Brazil.

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CONFLICT OF INTERESTS

The authors declare no conflict of interests.

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AUTHORS' CONTRIBUTIONS

Maria Joana Nunes de Azevedo: sample collection, laboratory processing, statistical analysis, manuscript writing; Suzana dos Santos Nunes: sample collection, laboratory processing, manuscript writing; Fabyanne Guimarães de Oliveira: sample collection, laboratory processing, manuscript writing; Danielle Albuquerque Pires Rocha: elaboration of the research project, manuscript writing.

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