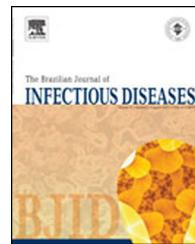The Brazilian Journal of
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Original article

Chlamydia trachomatis infection in a sample of northern Brazilian pregnant women: prevalence and prenatal importance

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ABSTRACT

There are limited data regarding prevalence of *Chlamydia trachomatis* infection among northern Brazilian pregnant women.

Objective: The purpose of this study was to estimate the prevalence of chlamydial infection among pregnant women in their third trimester and to determine the repercussion of this infection on their offspring.

Methods: In the first phase of this study 100 pregnant women receiving prenatal care in a local public university hospital were examined to assess the prevalence of genital *C. trachomatis* infection by polymerase chain reaction technique. In the second phase, 88 pregnant women were prospectively evaluated for premature rupture of membranes, puerperal consequences associated with chlamydial infection, and neonates were checked for low-birth weight.

Results: The prevalence rate of chlamydial infection was 11%, and 72.7% of the positive participants were predominantly less than 30 years of age ($p = 0.1319$). A total of 36.4% of the participants had premature rupture of membranes ($p = 0.9998$). Neither low-birth weight infants nor preterm delivery were observed. A cohort of 16 newborn babies were followed-up to 60 days of life to ascertain outcome: 50% had respiratory symptoms. Neonates born to infected mothers had a higher risk to develop respiratory symptoms in the first 60 days of life.

Conclusion: The scarcity of data about the effects of chlamydial infection on pregnancy and neonatal outcomes justified this study. Diagnosing and treating chlamydial infection during the third trimester of pregnancy may prevent neonate infection. Therefore, preventive screening should be seen as a priority for early detection of asymptomatic *C. trachomatis* infection as part of local public health strategies.

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Introduction

Chlamydia trachomatis infection is nowadays considered the most frequent sexually transmitted disease (STD) in the developed world. The World Health Organization (WHO) estimate that annually nearly 100 million new cases occur around the world.[1] Studies show that chlamydial infections is the commonest transmitted bacterial disease in European countries and in the United States with more than 2.8 million new cases estimated to occur each year.[2] During 2009, more than 1.2 million cases of chlamydial infection were reported to Centers for Diseases Control (CDC). Prevalence of chlamydial infection was 6.8% among sexually active females aged 14–19 years.[3]

According to the CDC, *C. trachomatis* infection among pregnant women in the United States is the third main cause of STD, with bacterial vaginosis and herpes simplex virus 2 being first and second, respectively.[3]

In Brazil, according to STDs epidemiologic studies in specific groups of women that attended gynecology, family planning, and prenatal clinics showed a prevalence of 2.1–27.1% for genital infection by *C. trachomatis*.[4] In the Amazon region, there is little data about the actual prevalence of chlamydial infections. Only AIDS, HIV in pregnant women and children, syphilis in pregnant women, and congenital syphilis have compulsory notification.[5]

In Manaus, the capital city of the state of Amazonas (AM), Brazil, Alencar et al. established a 27.1% prevalence of chlamydial infection by direct immunofluorescence technique.[6] On the other hand, Santos et al. and de Lima Freitas found 20.6% and 52.8% of sexually active women and infertile women, respectively, to be positive for *Chlamydia* using PCR technique.[7,8]

Many studies have provided estimates of chlamydial infection in pregnant women throughout the world. *C. trachomatis* disease, considered to be a treatable condition, continues to infect massive populations and the exact magnitude of this is still unknown.[9,10] Prevalence rates among pregnant women vary enormously around the world.[9,11] Jalil et al. conducted a study to estimate the prevalence of *C. trachomatis* infection in pregnant women from six Brazilian cities and found 9.4% using hybrid capture technique.[12] On the other hand, in Fortaleza, the capital city of Ceará, Brazil, Martins et al. found a prevalence of 11% in pregnant women by PCR technique.[13]

Uncomplicated *C. trachomatis* cervicitis in pregnancy may result in sporadic and recurrent miscarriage, preterm labor, premature rupture of membranes, low birth weight and postpartum endometritis.[14]

Maternal *C. trachomatis* infections have been associated with increased morbidity in newborns and infants up to three months of age. Approximately two thirds of neonates born vaginally to infected mothers will become infected at birth.[15] These infections can result in conjunctivitis, otitis media, pharyngitis and pneumonia in the newborn. Moreover, neonatal infection with *C. trachomatis* can cause long-term sequelae such as chronic obstructive pulmonary disease.[14]

The primary aim of this study was to assess the prevalence of *C. trachomatis* in the final trimester among pregnant women who attended the low-risk prenatal clinic of a public health university hospital in Manaus, Amazonas, Brazil.

In Manaus, there are no studies related to vertical transmission of *C. trachomatis*, so the secondary aim of this study was to ascertain possible neonatal implications associated to maternal *C. trachomatis* infection. Moreover, the purpose of this study was to evaluate *C. trachomatis* infection rate associated with socio-economic status of the participants enrolled in the study, adverse pregnancy outcomes associated with miscarriage, preterm labor, premature rupture of membranes and low birth weight.

Materials and methods

Endocervical samples were collected from 100 pregnant women receiving care at the low-risk prenatal clinic of Dona Francisca Mendes University Hospital in Manaus, Amazonas, Brazil, between January and June 2005. The first phase of this study was a cross-sectional evaluation of the prevalence of *Chlamydia* infection in the final trimester of pregnancy (as of 29 weeks of gestation). In the second phase of this study, pregnant women were prospectively followed to evaluate postpartum effects of this infection as well as upon their neonates up to sixty (60) days after birth.

This study was approved by the Human Research Ethics Committee at the Federal University of Amazonas and performed in accordance with the Declaration of Helsinki.

Assessment of patients

Pregnant women included in this study answered a standardized questionnaire to collect information on age, marital status and medical history, number of lifetime sexual partners and socio-economic status. Family income and education level were the criteria used to determine socio-economic status. Patients were excluded if the gestational age was less than 28 weeks and six days, had infections requiring antibiotic therapy or a history of antibiotic use in the preceding 30 days. In addition, pregnant women who had sexual intercourse in the past 72 h, history of vaginal bleeding or who used vaginal cream, suspected premature rupture of the membranes, and a diagnosis of placenta previa were also excluded.

In the first phase of the study all participants were contacted by telephone so that the researchers could collect information about their maternity admission and start the second phase of the study. During the second phase of the study 88 pregnant women who met the eligibility criteria had their delivery checked and were followed-up for 60 days after giving birth. Of a total of 100 participants in the first phase of the study 12 were excluded from the study: four could not be contacted and eight due to insufficient follow-up time (less than 40 days of postpartum).

A cohort study was carried out in 88 newborns who were evaluated in the first 24 h and monitored thereafter every 15 days up to 60 days of life. However, 12 neonates were excluded due to loss of contact (four) and to follow-up time less than 60 days of life (eight). Moreover, one case of stillbirth occurred. Subsequent evaluations were conducted by telephone and if some ocular discharge or respiratory symptoms appeared in the neonate a clinical evaluation was requested. Furthermore, patient's complaints, clinical evaluation and physical exams

were registered in the questionnaire and entered in Epi-Info data bank.

Collection of the samples

Endocervical material for smears was collected using a cytobrush and subsequently re-suspended in 400 μL of TE (10 mM Tris–HCl pH 8.0 and 1 mM EDTA) and stored at −20° C until DNA extraction.

Preparation of the samples for PCR

Each sample was supplemented with 400 μL TPK solution [TE, 20% Tween, 10 mg/mL proteinase K], followed by incubation at 56° C for 1 h and then boiling for 10 min. DNA from samples that contained blood was extracted by phenol/chloroform.

PCR amplification of C. trachomatis DNA plasmid

The KL1 (5′TCCGGAGCGAGTTACGAAGA3′) and KL2 (3′AATCAATGCCCGGGATTGGT5′) primers were used to amplify a 241 bp fragment of the *C. trachomatis* DNA plasmid by PCR. The PCR consisted of 5.0 μL of DNA; 5.0 μL of 10× PCR buffer; 2.0 μL of 50 mM $MgSO_4$; 1.0 μL of 10.0 mM dNTP; 5.0 μL of each primer (5 pmol/μL); 0.2 μL of 5 U/μL Taq DNA polymerase; and 26.8 μL Milli-Q water to complete the 50 μL volume. The amplification was performed in a PXE 0.2 Thermal Cycler (Thermo Electron Corporation), using the following program with 40 amplification cycles: pre-denaturing at 94 °C for 30 s, denaturation at 94 °C for 30 s, primer annealing at 64 °C for 30 s and primer extension at 68 °C for 2 min, followed by a final extension at 68 °C for 5 min.

DNA sequencing

To confirm the presence of 241 bp PCR products, we sequenced positive samples of *C. trachomatis* with the MegaBACE 1000 DNA Analysis System. The amplified products were digested with EXO and SAP enzymes, followed by incubation at 37 °C and 80 °C for 1 h and 30 min, respectively. The purified products were sequenced using DYEnamic ET DYE Terminator Cycle Sequencing kit for MegaBace from GE Healthcare Lifesciences according to manufacturer's protocol. The sequencing reactions were separated and analyzed on the automatic sequencer.

Statistical analysis

Statistical analyses were performed to calculate the absolute and relative frequency distributions for qualitative variables; as well as median and standard deviation values for quantitative variables. The data were analyzed to evaluate the association between the categorical variables and PCR results using the Pearson χ^2 test with the Yates correction or Fisher's exact test. To analyze the risk factors of the neonates relative risks (RR) were calculated. Student's *t*-test was used to compare means of normally distributed data. To estimate the prevalence of *C. trachomatis* in pregnant women a 95% confidence interval [CI] was calculated. Throughout this study, $p < 0.05$ was considered significant. Statistical analysis was

Table 1 – Speculum examination according to PCR results for *C. trachomatis* in pregnant women in Manaus, Amazonas, Brazil.

Speculum examination	PCR for *Chlamydia trachomatis*				Total
	Positive (n = 11)		Negative (n = 89)		
	n	%	n	%	
Cervicitis					0.0193
Yes	4	36.4	7	7.9	
No	7	63.6	82	92.1	
Endocervical secretion					0.0411
Yes	7	63.6	25	28.1	
No	4	36.4	64	71.9	

Table 2 – Premature rupture of membranes in pregnant women according to PCR results for *Chlamydia trachomatis* in Manaus, Amazonas, Brazil.

Premature rupture of membranes	PCR for *Chlamydia trachomatis*				Total
	Positive		Negative		
	n	%	n	%	
Yes	4	36.4	32	41.6	36
No	7	63.6	45	58.4	52
Total	11	12.5	77	87.5	88

performed using the Epi-Info version 3.3 software for Windows, developed and distributed by CDC, Atlanta, GA, USA.

Results

Phase I – study

Out of 100 patients 11 (11.0%) were *C. trachomatis*-positive by PCR. The mean age of PCR-positive patients was 23.5 years with a standard deviation of ±4.5 years.

Although we assessed associations between *C. trachomatis* infection with family income (45.5%) and their educational level (45.5%), no statistically significant association could be found.

Endocervical secretion and cervicitis were observed in 63.6% and 36.4%, respectively, among those women positive for *C. trachomatis* infection. Both endocervical secretion and cervicitis were significantly associated with *C. trachomatis* infection ($p = 0.0411$ and 0.0193, respectively) as shown in Table 1.

Phase II study – maternal and perinatal data

There was no significant association between positivity for *C. trachomatis* infection and premature rupture of membranes (Table 2). Neither preterm labor (less than 37 weeks gestational age) and nor low-birth weight (weight less than 2500 g/5.5 pounds) was observed among the PCR-positive women.

Cesarean delivery was indicated for PCR-positive primiparous pregnant woman. Chronic tubal sequelae were

Table 3 – Incidence of respiratory symptoms in a cohort of 87 neonates exposed according to maternal *Chlamydia trachomatis* infection in Manaus, Amazonas, Brazil.

Exposure to Chlamydia trachomatis	Respiratory symptoms				Total
	Yes		No		
	n	%	n	%	
Yes	8	50.0	2	2.8	10
No	8	50.0	69	97.2	77
Total	16	18.4	71	81.6	87

RR = 7.7; 95% CI (3.7–15.9).

Table 5 – Mode of delivery and respiratory symptoms of neonates in Manaus, Amazonas, Brazil.

Mode of delivery	Respiratory symptoms				Total
	Yes		No		
	n	%	n	%	
Cesarean section	7	43.8	29	40.8	36
Vaginal	9	56.2	42	59.2	51
Total	16	18.4	71	81.6	87

assessed by the surgery description. One week after birth a patient was readmitted to the hospital with diagnosis of postnatal endometritis. Another *C. trachomatis* positive participant gave birth to a stillborn fetus with 34 weeks gestation. After this episode, this patient developed postnatal endometritis.

Cohort study of neonatal outcome

Of the 87 newborns, 16 (18.4%) presented respiratory symptoms. Of these newborns, eight (50%) were born to *C. trachomatis*-positive mothers evidencing a RR of 7.7 (Table 3). Moreover, neonates born to positive mothers were more likely to develop respiratory symptoms such as nasal obstruction (RR = 6.7), coryza (RR = 7.7), cough (RR = 27.0) and dyspnea (RR = 15.4) (CI 95%) (Table 4). There was no statistically significant ($p = 0.8312$) association between *C. trachomatis* infection and type of delivery (cesarean section or vaginal) (Table 5).

Discussion

Studies show that *C. trachomatis* infection during pregnancy may lead to serious complications. Epidemiological data suggest that pregnant women may be a specific target group for *C. trachomatis* screening. Antenatal screening, as recommended by the CDC,[3] would be beneficial to decrease morbidity amongst women themselves, but also to prevent vertical (infant) and horizontal (partner) transmission.

Although studies have demonstrated prevalence of *C. trachomatis* in pregnant women using hybrid capture technique in Brazilian cities,[12] we decided to undertake this study using the conventional PCR technique. However, although hybrid capture technique is widely used for the detection of pathogens, it may lack sensitivity in comparison with PCR techniques.[16] PCR was the first technique to be developed and because of its ability to amplify specific targets and due to superior sensitivity it remains the most widely used molecular technique.[17]

The prevalence of *C. trachomatis* infection in this study was 11.0%. Although Jalil et al.[12] found a prevalence of 9.4% among Brazilian pregnant women, the rate identified in this study is in agreement with the findings of Martins et al.[13] (11.0%). However, as mentioned above there were no data on *C. trachomatis* infection among pregnant women in the city of Manaus before this study. The main goal of this study was to better understand the epidemiology of *Chlamydia* infection in this Brazilian city. As compared to other *C. trachomatis* infection prevalence studies conducted in Brazil, it is possible that the prevalence rate identified in this study is associated with the population characteristic. Although we found that the majority (45.5%) of the participants with PCR-positive results had a low family income, in addition to low educational level (45.5%), and had no knowledge of the existence of *C. trachomatis* and its effects on women's reproductive health. A total of 81.8% of PCR-positive *C. trachomatis* women in this study were married or were living a cohabitating situation. Only one lifetime sexual partner was reported by 90.9% of the women; 72.7% of the PCR-positive patients were under 25 years old. The risk of presenting chlamydial infection is two times higher in under 25 years old women.[18] In most of the studies the majority of positive participants were under 25 years old, which is could possibly be explained by the unhealthy lifestyle behavior of

Table 4 – Incidence of nasal obstruction, coryza, cough and dyspnea in a cohort of neonates according to maternal *Chlamydia trachomatis* infection up to 60 days after birth in Manaus, Amazonas, Brazil.

Exposure to Chlamydia trachomatis	Coryza[a]				Total	Nasal obstruction[b]				Total	Cough[c]				Total	Dyspnoea[d]				Total
	Yes		No			Yes		No			Yes		No			Yes		No		
	n	%	n	%		n	%	n	%		n	%	n	%		n	%	n	%	
Yes	5	50.0	5	6.5	10	7	46.7	3	4.2	10	7	77.8	3	3.8	10	2	66.7	8	9.5	10
No	5	50.0	72	93.5	77	8	53.3	69	95.8	77	2	22.2	75	96.2	77	1	33.3	76	90.5	77
Total	10		77	85.5	87	15	17.2	72	82.8	87	9	10.3	78	89.7	87	3	3.4	84	96.6	87

a RR = 7.7; 95% CI (2.7–22.0).
b RR = 6.7; 95% CI (3.1–14.6).
c RR = 27.0; 95% CI (6.5–112.2).
d RR = 15.4; 95% CI (1.2–565.5).

this age group, such as unprotected sex.[19] These factors may be due to high number of unrecognized infected men which provides a reservoir for spreading the infection to women via sexual contact.[20] These findings indicate the need for disseminating information about the risk factors for *Chlamydia* infection. The following factors may be responsible for the inadequate incorporation of preventive measures by women: low educational level of the population; and lack of screening for *Chlamydia* infection in the public health system.[21] Also, it is possible that the prevalence rate identified in this study is associated with low socio-economic status and low family income of the participants enrolled in this study. Women of low-income groups or of low educational level have no access to screening tests.

Considering that 36.4% of the PCR-positive patients had cervicitis identified at gynecological examination, this is an important clinical finding for women's reproductive health because in certain situations, cervicitis may ensue infection in pregnancy, which may result in preterm labor and neonatal infections. This clinical finding is in agreement with the syndromic approach protocol for vaginal discharge and cervicitis established by CDC.[22] Considering that chlamydial infections are usually asymptomatic and therefore difficult to screen, women without such marker would be considered to be at high risk of ascending infection, which may lead to severe complications resulting in tubal damage.[23]

In this study, we verified a high rate of abortion. In contrast to most studies on the subject, 30 (90.9%) of these participants were negative for *C. trachomatis* infection. Although various studies state that *C. trachomatis* may cause abortion in several animal species, endocervical *Chlamydia* infections may or may not be associated with spontaneous abortion.[24,25] This diagnostic accuracy is important because it has the potential to help identifying cases at higher risk for spontaneous abortion.

In this study, we verified a case of a pregnant woman diagnosed with *Chlamydia* infection developed a stillborn fetus. The putative association with the infection could not be confirm because the fetus was investigated. Andrews et al.[26] related a study where chlamydial infection detected at 24-week gestation could be associated with double risk of preterm birth. Yet, infection that was detected at 28-week gestation had no significant effect of preterm birth.

Also, some studies indicate that *C. trachomatis* is an important etiological agent of endometritis in women with concomitant signs of salpingitis.[27] It is possible that cases of postpartum endometritis in this study were due to *C. trachomatis* infection. Ascending infections of the female genital tract with sexually transmitted agents of the lower genital tract may occur during the postpartum period.[28]

In this study, 50% of positive *C. trachomatis* newborns presented respiratory problems. Various studies state that transmission of *C. trachomatis* from mothers to their newborns usually occurs at the time of delivery with passage of the newborn through the infected endocervix. According to Numazaki et al. and Schaad et al. the possibility of intrauterine infection at late pregnancy has also been reported.[29] Our finding showed a relation between *C. trachomatis* infection and respiratory symptoms. Cough symptoms showed to have an increased risk (RR 27.0) when neonates are born to

infected mothers, followed by dyspnea (RR 15.4), coryza (RR 7.7) and nasal obstruction (RR 6.7). Respiratory symptoms were more frequent in neonates born to *Chlamydia* infected mothers. In addition, in this study, respiratory symptoms such as cough, was the most important symptom found in infants. Therefore, it is clear that clinical findings in this study in neonates born to *Chlamydia* infected mothers were associated with antenatal chlamydial infection in their mothers. Bébéar and Barbeyrac have also described that untreated infection acquired at birth can persist for months or years.[30] Moreover, the role of chlamydial infection resulting in neonatal pneumonia has also been considered. Although mortality associated to neonatal pneumonia caused by chlamydial infection is low, sequelae such as impairment in lung function may be evidenced.[14] These findings indicate the great importance of a systematic investigation for this curable STD in pregnant women. This points to the necessity of an important clinical measure to reduce vertical transmission and complications of *C. trachomatis* infection and other causes of curable STD.

Conclusion

Our results demonstrate a true local reality, where women lack information about *Chlamydia* infection and an awareness of the possibility of gestational, perinatal and neonatal complications. Most of the time, pregnant women who are less privileged in socioeconomic and educational terms are left without any or insufficient medical assistance. It is important to emphasize the need for an equitable distribution of resources in health services. Therefore, it is essential to implement antenatal screening programs in public health service laboratories using the PCR method for detecting *Chlamydia* infection in this high risk population during the last month of pregnancy.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

REFERENCES

1. WHO. http://www.who.int/docstore/hiv/GRSTI/pdf/figure04.pdf (accessed 27.06.11).
2. Fenton KA, Lowndes CM. Recent trends in the epidemiology of sexually transmitted infections in the European Union. Sex Transm Infect. 2004;80:255–63.
3. CDC. Progress toward interruption of wild poliovirus transmission-worldwide, January 2007–April 2008. MMWR. 2008;57:489–94.

4. Miranda AE, Gadelha AMJ, Passos MRL. Impacto da Infecção pela *Chlamydia trachomatis* na Saúde Reprodutiva. DST – J Bras Doenças Sex Transm. 2003;15:53–8.

5. Sardinha JCG, Santos AR, Segadilha DAC. Plano Interinstitucional das DST e AIDS no Estado do Amazonas; 1999.

6. Alencar AAF, Ferreira LCL, Loureiro JAS. Detecção de *Chlamydia trachomatis* por imunofluorescência direta em esfregaços endocervicais. J Bras Ginecol. 1993;103:199–203.

7. Santos C, Teixeira F, Vicente A, Astolfi-Filho S. Detection of *Chlamydia trachomatis* in endocervical smears of sexually active women in Manaus-AM, by PCR. Braz J Infect Dis. 2003;2:91–5.

8. de Lima Freitas NS, Borborema-Santos CM, Barroso Serrão das Neves D, Costa de Oliveira CM, Dutra Ferreira JR, Astolfi-Filho S. High prevalence detection of *Chlamydia trachomatis* by polymerase chain reaction in endocervical samples of infertile women attending university hospital in Manaus–Amazonas, Brazil. Gynecol Obstet Invest. 2011;72:220–6.

9. Urdea M, Penny LA, Olmsted SS, et al. Requirements for high impact diagnostics in the developing world. Nature. 2006:73–9.

10. Ward BJ, Plourde P. Travel and sexually transmitted infections. J Travel Med. 2006;13:300–17.

11. Robinson AJ, Rogstad K. Adolescence: a time of risk taking. Sex Transm Infect. 2002;78:314–5.

12. Jalil EM, Pinto VM, Benzaken AS, et al. Prevalence of Chlamydia and *Neisseria gonorrhoeae* infections in pregnant women in six Brazilian cities. Rev Bras Ginecol Obstet. 2008;30:614–9.

13. Martins TA, Y-Bello P, Bello MD, et al. As Doenças Sexualmente Transmissíveis são Problemas entre Gestantes no Ceará? DST – J Bras Doença Sex Transm. 2004;16:50–8.

14. Mardh PA. Influence of infection with *Chlamydia trachomatis* on pregnancy outcome: infant health and life-long sequelae in infected offspring. Best Pract Res Clin Obstet Gynaecol. 2002;16:847–64.

15. Weinstock H, Dean D, Bolan G. *Chlamydia trachomatis* infections. Infect Dis Clin N Am. 1994;8:797–819.

16. Rodrigues AD, Cantarelli VV, Frantz MA, Pilger DA, Pereira FS. Comparison of hybrid capture and PCR for HPV detection in clinical samples. J Bras Patol Med Lab. 2009;45:457–62.

17. Sanguinetti M. New diagnostic tools. Haematol Rep. 2005;1:35–8.

18. Jackson Y, Sebo P, Aeby G, et al. Prevalence and associated factors for *Chlamydia trachomatis* infection among undocumented immigrants in a primary care facility in Geneva, Switzerland: a cross-sectional study. J Immigr Minor Health. 2010;12:909–14.

19. Land JA, Gijsen AP, Evers JLH, Bruggeman CA. *Chlamydia trachomatis* in subfertile women undergoing uterine instrumentation. Screen or treat? Hum Reprod. 2002;23:525–7.

20. Hamdad-Doudi F, Petit J, Eb F. Assessment of *Chlamydia trachomatis* infection in asymptomatic male partners of infertile couples. J Med Microbiol. 2004;53:985–90.

21. Ginige S, Fairley CK, Hocking JS, Bowden FJ, Chen MY. Interventions for increasing *Chlamydia* screening in primary care: a review. BMC Public Health. 2007;7:95.

22. Centers for Disease Control and Prevention. Diseases characterized by vaginal discharge. Sexually transmitted diseases treatment guidelines; 2010 http://www.cdc.gov/std/treatment/2010/vaginal-discharge.h (accessed 07.06.11).

23. Jha R, Vardhan H, Bas S, Salhan S, Mittal A. Cervical epithelial cells from *Chlamydia trachomatis*-infected sites coexpress higher levels of chlamydial heat shock proteins 60 and 10 in infertile women than in fertile women. Gynecol Obstet Invest. 2009;68:160–6.

24. Howie PW. Abortion and ectopic pregnancy. In: Whitfield CR, editor. Dewhurst's textbook of obstetrics and gynaecology for postgraduates. 5th ed. Oxford: Blackwell Science Ltd.; 1995. p. 140.

25. Quinn PA, Petric M, Barkin M, et al. Prevalence of antibody to *Chlamydia trachomatis* in spontaneous abortion and infertility. Am J Obst Gynaecol. 1987;156:291–6.

26. Andrews WW, Goldenberg RL, Mercer B, et al. The preterm prediction study: association of second-trimester genitourinary chlamydial infection with subsequent spontaneous preterm birth. Am J Obstet Gynecol. 2000;183:662–8.

27. Mårdh PA, Møller BR, Ingerslev HJ, Nüssler E, Weström L, Wølner-Hanssen P. Endometritis caused by *C. trachomatis*. Br J Vener Dis. 1981;57:191–5.

28. Temmerman M, Laga M, Ndinya-Achola JO, et al. Microbial aetiology and diagnostic criteria of postpartum endometritis in Nairobi, Kenya. Genitourin Med. 1988;64:172–5.

29. Numazaki K, Asanuma H, Niida Y. *Chlamydia trachomatis* infection in early neonatal period. BMC Infect Dis. 2003;3:2, http://dx.doi.org/10.1186/1471-2334-3-2.

30. Bébéar C, de Barbeyrac B. Genital *Chlamydia trachomatis* infections. Clin Microbiol Infect. 2009;15:4–10.