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Chlamydia trachomatis *infection in pregnant women with premature membrane rupture or premature delivery threat.*

Original Article

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SUMMARY.

Introduction. *Chlamydia trachomatis* is considered the causal agent of trachoma, salpingitis, endometritis, and may be involved in premature membrane rupture (PMR) and premature delivery threat (PDT). The aim of this work was to determine the presence of *C. trachomatis* antigens and antibodies against *C. trachomatis* in pregnant women with PMR, PDT or normal pregnancy (NP).

Material and methods. We took endocervical samples from 50 pregnant women of each group for determination of *C. trachomatis* antigens by means of an direct immunofluorescence method; additionally, 5 mL of peripheral blood were taken to identify anti-*C. trachomatis* antibodies by indirect immunoflurescence assay.

Results. Six per cent (3/50) of PMR patients showed *C. trachomatis* antigens and IgG anti-*C. trachomatis* antibodies. Two per cent (1/50) of PDT patients had *C. trachomatis* and IgM anti-*C. trachomatis* antibodies. Six per cent (3/50) of NP patients exhibited antigens *C. trachomatis* but no anti-*C. trachomatis* antibodies. Moreover, only IgG anti-*C. trachomatis* antibodies were found respectively in 10% (5/50), 10% (5/50) and 16% (8/50) of the PMR, PDT and NP women groups.

Conclusions. The finding of C. *trachomatis* antigens as well as anti-*C. trachomatis* antibodies in the three studied groups, emphasizes the importance of an opportune identification of the infection in order to apply the adequate treatment and prevent sequelae in both the pregnant women and their products.

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Key words: *Chlamydia trachomatis*, infection, pregnancy, premature membrane rupture, premature delivery threat.

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RESUMEN.

Chlamydia trachomatis en mujeres embarazadas con ruptura prematura de membranas o amenaza de parto prematuro.

Introducción. *Chlamydia trachomatis* se considera el agente causal de tracoma, salpingitis, endometritis y podría estar involucrada en la ruptura prematura de membrana (PMR) y amenaza de parto prematuro (PDT). El objetivo de este trabajo fue determinar la presencia de antígenos de *C. trachomatis* y anticuerpos contra *C. trachomatis* en mujeres embarazadas con PMR, PDT (ambos grupos de etiología desconocida) y mujeres con embarazo normal (NP).

Material y métodos. Se obtuvieron 50 muestras endocervicales por cada grupo de mujeres embarazadas, para la determinación de antígenos de *C. trachomatis*, por el método de inmunofluorescencia directa. Asimismo fueron tomados 5 ml de sangre venosa, para identificar la presencia de anticuerpos contra *C. trachomatis* por inmunofluorescencia indirecta.

Resultados. Seis por ciento (3/50) de las pacientes con PMR presentaron antígenos de *C. trachomatis* y anticuerpos IgG anti-*C. trachomatis*. Dos por ciento (1/50) con PDT tuvieron antígenos de *C. trachomatis* y anticuerpos IgM anti-*C. trachomatis*. Seis por ciento (3/50) de las pacientes con NP mostraron antígenos de *C. trachomatis*, pero no anticuerpos anti-*C. trachomatis*. Sin embargo, en 10% (5/50) , 10% (5/50) y 16% (8/50) con PMR, PDT o NP, respectivamente; solamente se encontraron anticuerpos IgG anti-*C. trachomatis*.

Conclusión. El hallazgo tanto de antígenos como anticuerpos anti-*C. trachomatis* en los tres grupos estudiados, resalta la importancia de la oportuna identificación de la infección, para la aplicación del tratamiento adecuado, para prevenir las secuelas de la infección, tanto en las mujeres embarazadas como en sus productos.

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Palabras clave: *Chlamydia trachomatis*, infección, embarazo, ruptura prematura de membranas, amenaza de parto prematuro.

INTRODUCTION.

Chlamydia trachomatis, an obligate intracellular bacterium, is one of the most common agents of sexually transmitted diseases in both industrialized and developing countries (1-4). In Mexico, there are no reliable official statistics, although diverse studies have been carried out to assess the extent of the problem (5-10). The prevalence of *C. trachomatis* infection in the Mexican population is variable depending on the level of attention and the clinical status of the patients (6,8,9).

The etiology of gestational and prenatal disturbances is diverse (8,11-14). The majority of these disturbances are related to bacterial infections of the urogenital tract (5,15-18). *C. trachomatis* infection has been associated with premature delivery threat, ectopic pregnancy and recurrent abortion (2,7,19-24). Non treatment of *C. trachomatis* infection in pregnant women can provoke conjunctivitis or pneumonia in the product at birth (19,21,25). The sequelae of *C. trachomatis* infection in the women include pelvic inflammatory disease (20,26,27) and infertility (23,28). The diagnosis of *C. trachomatis* infection is difficult since 70%-90% of the endocervical chlamydial infections in women are asymptomatic and may persist for months to years (1,29,30). The main identification procedures of *C. trachomatis* are cultures in *HeLa* or *McCoys* cells (24,31), fluorescent antibodies assay (32), enzyme immunoassays (EIA) (33), ligase chain reaction (LCR), polimerase chain reaction (PCR) (32,34-36) and genomic DNA analysis (37). This work had the objective of investigating to detect the presence of *C. trachomatis* antigens or antibodies against *C. trachomatis* in pregnant women with PMR or PDT by direct and indirect immunofluorescence methods respectively.

PATIENTS AND METHODS.

One hundred and fifty pregnant women receiving medical attention at the Gineco-Obstetric Hospital of the Medical National Center of Occident, IMSS, Mexico, were studied. Fifty women with PMR and fifty with PDT were chosen from the High Risk Pregnancy Service, without knowing the cause of their pathology. Fifty women with NP were from the Outpatient Service of the same Hospital. A clinical record was elaborated for each patient, which included age, number of pregnancies, sexual partners, weeks of gestation, abortion, perinatal infections. They were informed about the aims of the necessity of obtaining biological samples from them. Their approval was solicited and obtained.

Sample procedure. In accordance with the suppliers' instructions, we proceeded to obtain an endocervical sample. The cervix was cleaned with sterilized gauze. A large swab was introduced one centimeter into the endocervical channel and rotated 5-10 seconds to gently detach epithelial cells. The swab was withdrawn without touching the vaginal walls. All hemorrhagic and pustulous samples were discarded. Immediately after the sampling, two glass-slide frotis were set. The swab was rotated inside the circular mark on the glass-slide (bioMérieux 55331). All samples were fixed with acetone, and stored at –20°C until further processing. Besides, 5 ml of peripheral venous blood was also taken and the obtained serum was kept frozen.

Identification of *C. trachomatis* by direct immunofluorescence (DIF). In agreement with the equipment supplier we proceeded to determine *C. trachomatis*. All glass-slide endocervical samples were covered with 20 µl of monoclonal murine anti-*C. trachomatis* antibodies (bioMérieux 55321) and incubated at room temperature in a humid chamber for 15 min. Thereafter the glass-slide were washed twice with phosphate buffer saline (PBS) solution, drained and covered with glycerol-PBS and a coverslide. They were placed in darkness for an hour, and then observed with an epifluorescence microscope (40X). Samples exhibiting ten or more fluorescent chlamydial bodies (elemental or reticulate bodies) per field were declared positive. As a reference we used mammalian cells (negative control) or mammalian cells with chlamydial bodies (positive control) (bioMérieux 55321). The results were expressed qualitatively as number and percent of positive cases per group.

Determination of anti-*C. trachomatis* antibodies. The procedure was carried out in accordance with the equipment supplier's intructions. Anti-*C. trachomatis* antibodies were first evidenced by indirect immunofluorescence (IIF) as total immunoglobulines (Igs) (bioMérieux 75603). Positive samples with anti-*C. trachomatis* antibodies at > 1:124 dilution were processed to determine the class of antibody present in the samples (IgG bioMérieux 75692 or IgM bioMérieux 75692). The general procedure was the following: all pregnant women serum samples were diluted with PBS 1:124. Twenty µl of each sample were deposited on a glass-slide (bioMérieux 72051) containing *Chlamydia trachomatis*-Spot IF serotype L2 (prebound and inactivated antigen) and were incubated at 37°C in a humid chamber. The glass-slides were rinsed twice with PBS. Later, conjugated murine antibody against human immunoglobulin (Igs, IgG or IgM) was added to the first antibody. The glass-slides were reincubated, rinsed twice and mounted with a fixing solution. They were observed with an epifluorescense microscope with an objetive 40x. All samples displaying the "stared sky" aspect, with fluorescent green points over a red background at ≥ 1:124 dilution, were considered positive. The results were reported as number and percentage of positive cases per group.

Statistical analysis. Student's t test was used to compare means, with a "p" value <0.05 for

statistical significance.

RESULTS.

The background characteristics of the patients with PMR, PDT and NP are shown in table 1. Age, number of pregnancies, sexual partners and weeks of gestation, did not differ significantly among the groups. There was no association of these variables with the results of the determination of antigens of *C. trachomatis* or antibodies anti-*C. trachomatis* probably because of the small number of positive cases in the groups with PMR and PDT (table 3).

In table 2 it can be appreciated that out of a total 150 cases, only (7/150) 4.6% were positive to antigens of *C. trachomatis* whereas in the determination of antibodies anti-C. trachomatis there were (19/150) 12.6% positives cases. It is important to mention that the determination of antibodies anti-*C. trachomatis* revealed a higher prevalence of positives cases to *C. trachomatis.* Furthermore, determination of antibodies allowed to define the class of antibodies present in the positive cases (table 3), which in turn allowed to determine the evolution of the infection.

Table 3 shows that (3/50) 6% of the patients from the PMR group presented both *C. trachomatis* antigens and anti-C trachomatis IgG+ antibodies. These indicate the presence of an active infection. The single patient in the PDT group who presented *C. trachomatis* antigens and IgM+ antibodies; probably had a recent infection. Six percent (3/50) in the NP group only presented *C. trachomatis* antigens, a finding suggestive of an incipient infection. Finally 4% (2/50), 10% (5/50) and 16% (8/50) of women from groups PMR, PDT and NP respectively had only IgG+ anti-*C. trachomatis* antibodies. This could mean the presence of a chronic infection or an immunologic scar from previous contact with *C. trachomatis*.

Table 1
Main clinical characteristics of the pregnant women.

Parameters	PMR (n=50)	PDT (n=50)	NP (n=50)	p
Age (years)*	26.9±5.8	25.7±5.4	24.8±5.7	NS
Pregnancies*	2.9±2.1	2.4±1.4	2.4±1.7	NS
Abortion %	0	0	0	
Perinatal infection%	0	0	0	
Paterns*	1.02±0.1	1.04±0.01	1.06±0.2	NS
Gestation (weeks)*	31.9±2.3	33.3±3.4	34.4±2.3	NS

* mean ± S D. p = t "student".

Table 2
Determination of *C. trachomatis* antigens or antibodies in pregnant women.

Test	PMR (n=50)	PDT (n=50)	NP (n=50)	Total (n=50)
Antigen + DIF	3	1	3	7 (4.6%)
Antibodies + IIF	5	6	8	19 (12.6%)

DISCUSSION.

The study of *C. trachomatis* infections in women can be focused in different ways. On one hand, the risk factors propitiating the onset of the infection such as age, beginning of sexual life, socioeconomic level and sexual partners can be studied (9,38). On the other hand, sequelae of chlamydial infections in women are considered risk factors for the development of other gynaecologic pathologies, such as pelvic inflammatory disease, ectopic pregnancy, and infertility (8,20,26-28).

Table 3
Presence of *C. trachomatis* antigens and/or anti-*C. trachomatis* antibodies in pregnant women.

Infection Chlamydial	Antigens Antibodies	PMR (n=50)	PDT (n=50)	NP (n=50)
Active	Ag + IgG+	3(6%)	0	0
Chronic/past	Ag – IgG+	2(4%)	5(10%)	8(16%)
Reactive / incipient	Ag + IgG –	0	0	3(6%)
Recient	Ag + IgM+	0	1(2%)	0

Ag = antigen.

Opportune detection of *C. trachomatis* infection during pregnancy will allow effective treatment in order to avoid complications such as abortion (23), a low-product-weight (8,10,14), and a premature delivery threat (12,18) as well as transmission of the infection to the newborn during the passage through the infected cervix (13,17,39).

At present, a controversy persists about the most effective method to identify *C. trachomatis* (1,6,39-44) given the differences in the sensibility and specificity of molecular methods, fluorescent antibody assays and enzyme immunoassays(40-52). However, the cellular culture for *C. trachomatis* is a highly specific method and is considered as the "gold standard" test (1). It is opportune to mention that the course of *C. trachomatis* infection can be monitored through the determination of the antigen or the bacterium itself, as well as through anti-*C. trachomatis* antibodies.

The purpose of this work was to investigate the correlation between *C. trachomatis* infection and PDT or PMR in patients without knowning the cause of their pathology. The results showed a weak association between the infection and PMR or PDT groups. Six per cent and 2% of the woman included in these groups presented *C. trachomatis* antigens and anti-*C trachomatis* antibodies (IgG or IgM). Moreover, 4% of the group of PMR and 10% of the group PDT only presented anti-*C. trachomatis* antibodies (IgG). A connection between *C. trachomatis* infection and PMR and PDT has been suggested but up to now there is no formal data to support this association(13,14,17,18,23,26).

It has been observed that the other cervical pathologies, increase the risk of *C. trachomatis* infection, since the alterations in the columnar epithelium facilitate the advance of the elementary bodies and hence the establishment of the infection(1,12,13). However, our patients with PMR or PDT did not showed mycosis, bacterial infections, or other diseases associated with PMR or PDT. Actually, the lack of a percise diagnosis in these patients prompted us to think that the infection by *C. trachomatis* could be responsible; however, the number of studied cases did not allow the establishment of a satisfactory conclusion.

On the other hand, the number of women with NP positive to *C. trachomatis* (3/50 Ag+, 8/50 IgG+ anti-*C. trachomatis*) makes it advisable to continue with studies of opportune diagnosis of the infection, and to follow up on these women, with the purpose of establishing the effective treatment to prevent the complications originated by the infection by *C. trachomatis*.

REFERENCES.

1.- Center for Disease Control and Prevention: Recomendations for the prevention and manegement of *Chlamydia trachomatis* infections. MMWR 1993; 42:1-41.

2.- Schachter J, Jones RB, Butler RC, Rice B, Brooks D, van der Pol B, *et al*. Evaluation of the Vidas *Chlamydia* Test to detect and verify *Chlamydia trachomatis* in urogenital specimens. J Clin Microbiol 1997; 35:2102-06.

3.- Suchland KL, Counts JM, Stamm WE. Laboratory methods for detection of *Chlamydia trachomatis*: Survey of laboratories in Washington State. J Clin Microbiol 1997; 35:3210-14.

4.- Gaydos CA, Crotchfelt KA, Howell MR, Kralian S, Hauptman P. Quinn TC. Molecular amplification assays to detect chlamydial infections in urine specimens from high school female students and to monitor the persistence of chlamydial DNA after therapy. J Infect Dis 1998; 177:417-24.

5.- Narcio-Reyes ML, Solórzano-Santos F, Arredondo-García JL, Calderón-Jaimes E, Beltrán-Zúñiga M. Etiología de la infección cérvicovaginal en pacientes embarazadas y no embarazadas. Ginec Obstet Mex 1989; 57:41-6.

6.- Guerra-Infante F, Flores-Medina S, López-Hurtado M, Sosa-González IE, Arredondo-García JL. Evaluación de la sensibilidad y especificidad de tres reactivos de inmunofluorescencia directa para el diagnóstico de *Chlamydia trachomatis*. Ginec Obstet Mex 1994; 63:368-73.

7.- Bustos-López H, Barrón-Vallejo J, García-Malváez B, Kably-Ambe A, Cáceres-Zelaya H. Aplicación de un algoritmo diagnóstico prospectivo para pacientes con pérdida fetal recurrente. Ginec Obstet Mex 1995;

63:96-101.

8.- Bustos-López HH, Vázquez-Juárez ME, Arredondo-García JL, Lira-Plascencia J, Beltrán-Zúñiga M, Guerra-Infante F. Prevalencia de *Chlamydia trachomatis* en pacientes con esterilidad y embarazos no complicados. Perinatol Reprod Hum 1995; 9:227-34.

9.- Acosta-Cázares B. *Chlamydia trachomatis* como factor de riesgo de algunas complicaciones del embarazo. Enf Infec Microbiol 1996; 16:142-5.

10.- Díaz-Barreiro G, Díaz-López E, Servín-Ramírez JF. Frecuencia de *Chlamydia trachomatis* en el cérvix de pacientes embarazadas en control prenatal. Ginec Obstet Mex 1997; 65:48-51.

11.- Pastorek II JG. Infections during pregnancy. Clin Obst Gynecol 1993; 4:793.

12.- Scott JR. Recurrent miscarriage Overview and recomendations. Clin Obstet Gynecol 1994; 37: 768-73.

13.- Numazaki K, Kusaka T, Chiba S. Perinatal complications are associated with seropositivity to *Chlamydia trachomatis* during pregnancy. Clin Infect Dis 1996; 23:208.

14.- Bajares M. Infecciones ginecológicas por Clamidia. Rev Obstet Ginecol Venez 1996; 56:S11-16.

15.- Carey JC, Yaffe SJ, Catz C. The vaginal infections and prematurity study: An review. Clin Obstet Gynecol 1993; 36:809-20.

16.- Holst E, Goffeng AR, Andersch B. Bacterial vaginosis and vaginal microorganisms in idiopathic premature labor and association with pregnancy outcome. J Clin Microbiol 1994; 32:176-86.

17.- Gencay M, Koskiniemi M, Saikku P, Puolakkainen M, Raivio K, Koskela P, *et al*. *Chlamydia trachomatis* seropositivity during pregnancy is associated with perinatal complications. Clin Infec Dis 1995; 21:424-6.

18.- Nadisauskiene R, Bergström S, Stankeviciene I, Spukaite T. Endocervical pathogens in women with preterm and term labour. Gynecol Obstet Invest 1995; 40:179-82.

19.- West SK, Muñoz B, Lynch M, Kayongoya A, Mmbaga BBO, Taylor HR. Risk factors for constant, severe trachoma among preschool children in Kongwa, Tanzania. Am J Epidemiol 1996; 143:73-8.

20.- Hillis SD, Owens LM, Marchbanks PA, Amsterdam LE, Mac Kenzie WR. Recurrent chlamydial infections increase the risks of hospitalization for ectopic pregnancy and pelvic inflammatory disease. Am J Obstet Gynecol 1997; 176:103-7.

21.- Tasdemir I, Tasdemir M, Kodama H, Sekine K, Tanaka T. Relationship of chlamydial infection to male infertility: Sperm parameters / antisperm antibodies. Arch STD/HIV Res 1995; 9:13-7.

22.- Mehanna MTR, Rizk MA, Eweiss NYM, Ramadan M, Zaki SA, Sadek A, *et al*. Chlamydial serology among patients with tubal factor infertility and ectopic pregnancy in Alexandria, Egypt. Sex Transm Dis 1995; 22:317-21.

23.- Witkin SS, Ledger WJ. Antibodies to *Chlamydia trachomatis* in sera of women with recurrent spontaneous abortions. Am J Obstet Gynecol 1992; 167:135-9.

24.- Shuland RJ, Rockey DD, Bannantine JP, Stamm WE. Isolates of *Chlamydia trachomatis* that occupy nonfusogenic iclusions lack incA, a protein localized to the inclusion membrane. Infect Immun 2000; 68:360-7

25.- Ghaem-Maghami S, Bailey RL, Mabey DCW, Hay PE, Mahdi OSM, Joof HM, *et al*. Characterization of B-cell responses to *Chlamydia trachomatis* antigens in humans with trachoma. Infec Immun 1997; 65:4958-64.

26.- Faro S. *Chlamydia trachomatis*: Female pelvic infection. Am J Obstet Gynecol 1991; 164:1767-70.

27.- Kimani J, Maclean IW, Bwayo JJ, MacDonald K, Oyugi J, Maitha GM, *et al*. Risk factors for *Chlamydia trachomatis* pelvic inflammatory disease among sex workers in Nairobi, Kenya. J Infec Dis 1996; 173:1437-44.

28.- Toye B, Laferrière C, Claman P, Jessamine P, Peeling R. Association between antibody to the chlamydial heat-shock protein and tubal infertility. J Infec Dis 1993; 168:1236-40.

29.- Toye B, Peeling RW, Jessamine P, Claman P, Gemmill I. Diagnosis of *Chlamydia trachomatis* infections in asymptomatic men and women by PCR assay. J Clin Microbiol 1996; 34:1396-400.

30.- Kacena KA, Quinn SB, Howell MR, Madico GE, Quinn TC, Gaydos CA. Pooling urine sample for ligase chain reaction screening for genital *Chlamydia trachomatis* infection in asymptomatic women. J Clin Microbiol 1998;

36:481-5.

31.- Pate MS, Ed M, Hook III EW. Laboratory to laboratory variation in *Chlamydia trachomatis* culture practices. Sex Transm Dis 1995; 22:322-6.

32.- Dean D, Ferrero D, McCarthy M. Comparison of performance and cost-effectiveness of direct fluorescent-antibody, ligase chain reaction, and PCR assay for verification of chlamydial enzyme immunoassay results for populations with a low to moderate prevalence *of Chlamydia trachomatis* infection. J Clin Microbiol 1998; 36:94-9.

33.- Chan EL, Brandt K, Horsman G. Evaluation of Sanofi Diagnostics Pasteur *Chlamydia* Microplate EIA shortened assay and comparison with cell culture and Syva *Chlamydia* MicroTrak II EIA in high- and low-risk populations. J Clin Microbiol 1995; 33:2839-41.

34.- Hook EW, Smith K, Mullen C, Stephens J, Rinehardt L, Pate MS, Lee HH. Diagnosis of genitourinary *Chlamydia trachomatis* infections by using the ligase chain reaction on patient-obtained vaginal swabs. J Clin Microbiol 1997; 35:2133-5.

35.- Puolakkainen M, Hiltunen-Back E, Reunala T, Suhonen S, Lähteenmäki P, Lehtinen M, *et al*. Comparison of performances of two commercially available tests, a PCR assay and a ligase chain reaction test, in detection of urogenital *Chlamydia trachomatis* infection. J Clin Microbiol 1998; 36:1489-93.

36.- Carroll KC, Aldeen WE, Morrison M, Anderson R, Lee D, Mottice S. Evaluation of the Abbott LCx ligase chain reaction assay for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in urine and genital swab specimens from a sexually transmitted disease clinic population. J Clin Microbiol 1998; 36:1630-3.

37.- Morré SA, Ossewaarde JM, Lan J, van Doornum GJJ, Walboomers JMM, MacLaren DM, *et al*. Serotyping and genotyping of genital *Chlamydia trachomatis* isolats reveal variants of serovars Ba, G, and J as confirmed by omp1 nucleotide sequence analysis. J Clin Microbiol 1998; 36:345-51.

38.- Lin JSL, Donegan SP, Heeren TC, Greenberg M, Flaherty EE, *et al*. Transmission of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* among men with urethritis and their female sex partners. J Infect Dis 1998; 178:1707-12.

39.- Numazaki K, Niida Y, Chiba S. Antigen detection of *Chlamydia trachomatis* from the endocervix is not enough for screening of perinatal complications. Am J Obstet Gynecol 1997; 176:951-2.

40.- van der Pol B, Williams JA, Jones RB. Rapid antigen detection assay for identification of *Chlamydia trachomatis* infection. J Clin Microbiol 1995; 33:1920-1.

41.- Goessens WHF, Mouton JW, van der Meijden WI, Deelen S, van Rijsoort-Vos TH, Lemmens-den TN, *et al*. Comparison of three comercially available amplification assays, AMP CT, LCx, and Cobas Amplicor, for detection of *Chlamydia trachomatis* in first –void urine. J Clin Microbiol 1997; 35:2628-33.

42.- Lauderdale TL, Landers L, Thorneycroft I, Chapin K. Comparison of the PACE 2 assay, two amplification asays, and clearview EIA for detection of *Chlamydia trachomatis* in famele endocervical and urine specimens. J Clin Microbiol. 1999; 37:2223-9.

43.- Stam WE. Chlamydia trachomatis infections: progress and problems. J Infect Dis 1999; 179: S380-3.

44.- Green TA, Black CM, Johnson RE. Evaluation of bias in diagnostic-test sensitivity and specificity estimates computed by discrepant analysis. J Clin Microbiol 1998; 36:375-81.

45.- Crotchfelt KA, Pare B, Gaydos C, Quinn TC. Detection of *Chlamydia trachomatis* by the gen-probe amplified *Chlamydia trachomatis* assay (AMP CT) in urine specimens from men and women and endocervical specimens from women. J Clin Microbiol 1998; 36:391-4.

46.- Fong CKY, Falcone J, Landry ML. Use of a single swab in multi-microbe or flex trans transport medium for detection of *Chlamydia trachomatis* by Roche Amplicor PCR and culture in specimens for two different patient populations. J Clin Microbiol 1997; 35:2427-9.

47.- Peeling RW, Bailey RL, Conway DJ, Holland MJ, Campbell AE, Jallow O, *et al*. Antibody response to the 60-kDa chlamydial heat-shock protein is associated with scarring trachoma. J Infect Dis 1998; 177:256-9.

48.- Chan EL, Brandt K, Horsman GB. A 1-year evaluation of Syva Micro Trak Chlamydia enzyme immunoassay with selective confirmation by direct fluorescent-antibody assay in high-volume laboratory. J Clin Microbiol 1994; 32:2208-11.

49.- Haddix AC, Hillis SD, Kassler WJ, The cost

effectiveness of azithromicyn for *Chlamydia trachomatis* infections in women. Sex Transm Dis 1995; 22:274-80.

50.- Johnson AM, Grun L, Haines A. Control de la infección genital por *Chlamydia.* BMJ 1997; 5:9-11.

51.- Kapala J, Copes D, Sproston A, Patel J, Jang D, Petrich A, *et al*. Pooling cervical swabs and testing by ligase chain reaction are accurate and diagnosis of *Chlamydia trachomatis*. J Clin Microbiol 2000; 2480-3.

52.- VanDerpol B, Quinn TC, Gaydos CA, Crotchfelt K, Schachter J, Moncada J, *et al*. Multicenter evaluation of the AMPLICOR and automated COBAS AMPLICOR CT/ NG tests for detection of *Chlamydia thrachomatis*. J Clin Microbiol 2000; 38:1105-12.