Original article

Rapid assessment of sexually transmitted diseases in a sentinel population in Thailand: prevalence of chlamydial infection, gonorrhoea, and syphilis among pregnant women—1996

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Objective: To determine the prevalence of sexually transmitted diseases (STDs) among pregnant women in Thailand, where case reporting suggests a marked decrease in STDs following a campaign promoting condom use during commercial sex.
Design: Cross sectional study of women at their first visit to the study hospitals' antenatal clinics in Chiang Rai (n=500) and Bangkok (n=521).
Methods: First catch urine specimens were tested for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* using the Amplicor CT/NG polymerase chain reaction assay. Syphilis and HIV serological testing were performed in the study hospitals' laboratories.
Results: The prevalence of chlamydial infection was 5.7%, gonorrhoea 0.2%, and syphilis 0.5% (all VDRL or RPR titres were ≤1:4). The prevalence of HIV infection was 7.1% in Chiang Rai and 2.9% in Bangkok. In a multivariate logistic regression analysis, chlamydial infection was associated with younger age and with higher gestational age at first antenatal clinic visit, but was not associated with marital status, gravidity, city of enrolment, or HIV infection status.
Conclusions: There was a low prevalence of gonorrhoea and syphilis among these pregnant women in Thailand. Chlamydial infection was detected at a higher prevalence, especially among younger women and women registering later for antenatal care. Testing of pregnant women using easily collected urine specimens and a sensitive nucleic acid amplification assay is a feasible method of rapidly assessing chlamydial and gonococcal prevalence.
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Keywords: sexually transmitted disease surveillance; Thailand; pregnancy

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Introduction

Surveillance of sexually transmitted diseases (STDs) is necessary to estimate the burden of disease, allocate resources effectively, and evaluate control strategies. However, accurate surveillance is difficult. STDs are frequently asymptomatic (especially among women), specimen collection can be difficult, laboratory methods needed for accurate diagnosis are complicated and often unavailable in resource poor settings, and stigmatisation inhibits healthcare seeking and reporting. With these limitations to case reporting, periodic assessment of the prevalence of STDs in sentinel populations may be a useful alternative surveillance method.

Pregnant women seeking routine antenatal care constitute a well defined population and are a good choice for STD prevalence monitoring, allowing comparisons both across different geographic areas and over time in one area, without some of the biases involved in STD case reports or in the determination of STD prevalence among commercial sex workers or STD clinic patients.[1 2] The composition of these latter groups may vary widely in different settings. Also, the complications of STDs in pregnancy, such as congenital syphilis, ophthalmia neonatorum, and postpartum endometritis, may be prevented by antenatal diagnosis and treatment.

Newly developed, accurate nucleic acid amplification assays are able to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in urine specimens, making it feasible to detect these infections in settings where pelvic examination and cervical sampling are not routinely performed.[3–8] In this study of STDs in pregnant women in Thailand, we used urine specimens and the Amplicor CT/NG polymerase chain reaction (PCR) assay (Roche Molecular Systems, Branchburg, NJ, USA). Prior studies in non-pregnant women have shown the Amplicor PCR to be 92–97% sensitive and over 99% specific using urine specimens to detect *C trachomatis*.[3 6] This compared with a 72–86% sensitivity for cervical culture in those studies, both of which used a positive endocervical culture and/or a positive second amplification assay of a different nucleic acid target as a secondary gold standard. The Amplicor PCR for *N gonorrhoeae* is less well characterised. In two small studies using urine samples from non-pregnant women, the sensitivity was 71% (five of seven detected) to 94% (17 of 18 detected), and the specificity was 99%.[9 10]

Thailand experienced an explosive epidemic of heterosexually transmitted human immunodeficiency virus (HIV) infection beginning in the late 1980s, with the highest rates of infection in the upper northern part of the country.[11] Recognising the central role of commercial sex

Table 1 Demographic and obstetric characteristics of study participants at their first antenatal clinic visit, Thailand, 1996

Characteristic	*No*	*%*
Study site		
Chiang Rai	500	49
Bangkok	521	51
Age (years)		
⩽19	167	16
20–24	338	33
25–29	285	28
⩾30	231	23
Marital status		
Married	989	97
Unmarried	32	3
Gravidity		
Primigravida	428	42
Multigravida	591	58
Prior miscarriage, abortion, stillbirth		
Any	212	21
None	807	79
Trimester		
First	414	42
Second	367	37
Third	205	21

in the epidemic,[11–13] the Thai Ministry of Public Health implemented a nationwide programme in 1991 to promote condom use during commercial sex.[14 15] The programme included education and condom distribution, and was enforced by police sanctions against establishments in which STD were diagnosed among sex workers or their clients.[14 15] There have since been encouraging increases in condom use during commercial sex in Thailand,[15 16] and declines in STD case reports from STD clinics,[15] in rates of male patronage of female sex workers,[16] and in HIV infection rates among military conscripts.[16]

To ascertain the burden of STDs in Thailand in the setting of these changes, and to assess the feasibility of testing pregnant women using urine specimens and a nucleic acid amplification assay as a method of sentinel surveillance for *C trachomatis* and *N gonorrhoeae*, we determined the prevalence of these pathogens and of syphilis among pregnant women in Bangkok in central Thailand and in Chiang Rai, Thailand's northernmost province.

Methods

Women were enrolled at two hospitals in Bangkok—Siriraj Hospital, a university teaching hospital with the largest number of deliveries in the country (18 000 per year), and Rajavithi Hospital, the largest public maternity hospital in the country (17 000 births per year); and at Chiang Rai Hospital, a public hospital with the largest number of deliveries (5000 per year) in Chiang Rai. Pregnant women registering for their first visit to the study hospitals' antenatal care clinics during their current pregnancy were eligible for enrolment. To assure informed consent, women who could not speak and understand spoken Thai or who were less than 18 years old and were unmarried or unaccompanied by a parent or legal guardian were not eligible. Women who had already provided a urine specimen at their first clinic visit for routine dipstick testing before being instructed in proper specimen collection by the study staff were not enrolled. All eligible women were offered enrolment at two of the hospitals. At Siriraj Hospital every second group of approximately 10 eligible women was offered enrolment. Women were enrolled between June and December 1996 in Chiang Rai and during a 2 week period at each Bangkok hospital in November and December 1996. Written, informed consent was obtained from all study subjects or their parents or guardians. The study protocol was approved by the Thai Ministry of Public Health ethics review committee and an institutional review board of the US Centers for Disease Control and Prevention (CDC).

Each participant provided a 30 ml first catch urine specimen which was immediately cooled on wet ice in the clinic. In Chiang Rai, specimens were stored at 4°C for as long as 1 week before being shipped by overnight bus on wet ice to the HIV/AIDS Collaboration laboratory in Nonthaburi, a suburb of Bangkok. Specimens were brought from the Bangkok hospitals to the HIV/AIDS Collaboration laboratory on wet ice on the day of collection. Specimens were then stored at −70°C before shipping on dry ice to the CDC, Atlanta, where Amplicor CT/NG PCR was performed according to the manufacturer's instructions. The assay contained an internal control to test for the presence of PCR inhibitors.[17]

Counselling and voluntary serological testing for HIV infection and for syphilis were offered to all clinic attendees as part of routine antenatal clinic practice and testing was performed in the study hospitals' laboratories. In Chiang Rai, specimens that were positive on two different manufacturers' HIV enzyme immunoassays (EIA) were considered HIV positive; at Siriraj Hospital, specimens positive on one EIA and a particle agglutination test or a second EIA were considered positive; and at Rajavithi Hospital, specimens were considered HIV positive if they were positive on repeated EIA and on western blot. In Chiang Rai and at Siriraj Hospital the Venereal Disease Research Laboratory (VDRL) test was used for syphilis screening. At Rajavithi Hospital a rapid plasma reagin (RPR) test was used. Confirmatory treponemal tests (fluorescent treponemal antibody absorbed test (FTA-Abs) or *Treponema pallidum* haemagglutination assay (TPHA)) were performed routinely at Siriraj Hospital, but only if requested by the clinic physician at a subsequent clinic visit at the other two hospitals. Sexual risk behaviour data were not obtained. Customarily, women who were not legally married but were living with their partner reported that they were married. Treatment for STDs at the first or second antenatal clinic visit was ascertained by medical record review.

Urine PCR assay results were reported back to the clinics, and women with positive tests were contacted by telephone or by mail for follow up. They and their sex partners were treated according to established guidelines.[18] If the result was received post partum, infants born to infected mothers were referred for evaluation and treatment. At the clinic visit when treatment was initiated a second, follow up urine specimen was collected for repeat

Table 2 Prevalence of sexually transmitted diseases among pregnant women at their first antenatal clinic visit, Thailand, 1996

	Chiang Rai			*Bangkok*			*Total*		
		Positive			*Positive*			*Positive*	
Condition	*No*	*No*	*% (95% CI)*	*No*	*No*	*% (95% CI)*	*No*	*No*	*% (95% CI)*
*C trachomatis**	500	23	4.6 (3.0–6.7)	521	35	6.7 (4.8–9.1)	1021	58	5.7 (4.4–7.2)
*N gonorrhoeae**	500	2	0.4 (0.1–1.3)	521	0	0 (0.0–0.6)	1021	2	0.2 (0.0–0.6)
Syphilis†	493	3	0.6 (0.2–1.6)	519	2	0.4 (0.1–1.3)	1012	5	0.5 (0.2–1.1)

CI = mid-p confidence interval.
*Determined using a first catch urine specimen and Amplicor CT/NG polymerase chain reaction assay (Roche Molecular Systems).
†Positive non-treponemal test (VDRL or RPR) and either a positive confirmatory treponemal test (FTA-Abs or TPHA, three women) or no confirmatory treponemal test (two women). All VDRL and RPR test titres were ⩽1:4.

Amplicor CT/NG PCR testing. If the follow up urine specimen *C trachomatis* PCR assay was negative, *C trachomatis* ligase chain reaction (LCR, Abbott Laboratories, Chicago, IL, USA) was performed with that specimen according to the manufacturer's instructions.

Results

Of 1066 potential participants, 1021 (96%) were enrolled. Reasons for not enrolling included inability to produce a urine specimen (13 women); being less than 18 years old and unmarried or unaccompanied by a parent or guardian (eight); having already produced a urine specimen in the clinic before being instructed in proper specimen collection by the study staff (six); refusal to participate (four); and inability to speak Thai (two). The 45 non-participants were younger than the study participants (36% of non-participants *v* 16% of participants were age ⩽19 years); they were more likely to be unmarried (22% *v* 3%); and they had a higher median gestational age (19 weeks *v* 15 weeks).

Most study participants were 20–29 years old, married, and multigravida (table 1). The median age was 25 years (range 13–46 years). Of the 32 unmarried women 18 were single, nine divorced, and five widowed. In Bangkok, 250 women were enrolled at Siriraj Hospital and 271 at Rajavithi Hospital.

The overall prevalence of chlamydial infection was 5.7% and gonorrhoea 0.2%, with non-significant differences between the two study cities (table 2). The PCR assay was performed a mean of 40 days following specimen collection (range 12–97 days). As determined by the internal control, none of the specimens contained PCR inhibitors. None of the study participants was diagnosed with or treated for chlamydial infection or gonorrhoea at her first or second antenatal clinic visit, before the PCR test results were available—that is, all of the chlamydial and gonococcal infections identified in this study were subclinical. Seven women had a positive non-treponemal syphilis test (VDRL or RPR), but two of them had a negative confirmatory treponemal test (FTA-Abs or TPHA), yielding a total of five women (0.5%) with serological evidence of syphilis (three of the five had a positive confirmatory test and two did not have confirmatory testing). All five had low non-treponemal test titres; one was 1:4 and two each were 1:1 and 1:2. The prevalence of HIV infection among study participants was 7.1% in Chiang Rai (35 of 492 tested) and 2.9% in Bangkok (15 of 519 tested).

Of the 58 women with positive *C trachomatis* PCR assay results, 41 women (71%) returned to the clinic and had a second, follow up urine specimen collected at the time treatment was initiated. Of these 41 women, 33 (80%) had a positive second PCR assay for *C trachomatis* and for eight (20%) women this follow up test before treatment was negative. These eight *C trachomatis* PCR negative follow up specimens were also negative by *C trachomatis* LCR, and all eight of these women were from Chiang Rai. These negative follow up tests were not associated with a longer interval between initial and follow up tests; the 33 positive follow up tests were performed a median of 81 days following the initial positive test, while the eight negative follow up tests were performed a median of 61 days following the initial positive test. Both of the women with initial positive *N gonorrhoeae* PCR assays had negative follow up *N gonorrhoeae* PCR assays, performed 51 and 75 days after the initial test.

Chlamydial infection was associated with young age (⩽19 years) and with higher gestational age (second or third trimester) at first antenatal clinic visit, and was not significantly

Table 3 Risk factors for chlamydial infection among pregnant women at their first antenatal clinic visit, Thailand, 1996

		Chlamydia PCR Positive			
Characteristic	*No*	*No*	*%*	*Crude odds ratio (95% CI)*	*Adjusted odds ratio (95% CI)†*
Study site					
Bangkok	521	35	6.7	1.5 (0.8–2.7)	1.5 (0.9–2.7)
Chiang Rai	500	23	4.6	Referent	Referent
Age (years)					
⩽19	167	21	12.6	3.2 (1.4–7.5)	2.6 (1.4–5.0)
20–24	338	15	4.4	1.0 (0.4–2.5)	Referent
25–29	285	12	4.2	1.0 (0.4–2.5)	Referent
⩾30	231	10	4.3	Referent	Referent
Marital status					
Married	989	55	5.6	Referent	Referent
Unmarried	32	3	9.4	1.8 (0.3–5.9)*	1.0 (0.3–3.5)
Gravidity					
Primigravida	428	32	7.5	1.8 (1.0–3.1)	1.3 (0.6–2.7)
Multigravida	591	26	4.4	Referent	Referent
Prior miscarriage, abortion, stillbirth					
Any	212	11	5.2	0.9 (0.4–1.8)	1.3 (0.6–3.0)
None	807	47	5.8	Referent	Referent
Trimester					
First	414	12	2.9	Referent	Referent
Second	367	27	7.4	2.7 (1.3–5.6)	2.2 (1.1–4.6)
Third	205	17	8.3	3.0 (1.3–6.9)	2.6 (1.2–5.6)
HIV infection status					
Seropositive	50	4	8.0	1.5 (0.4–4.2)*	1.4 (0.5–4.2)
Seronegative	960	54	5.6	Referent	Referent

PCR = polymerase chain reaction assay, CI=confidence interval.
*Exact confidence limits.
†Multivariate logistic regression with all variables shown in table 3.

associated with HIV infection (table 3). A targeted screening of only women who were age ⩽19 years would have resulted in the highest yield per test (12.6% positive), but would have identified only 36% of the chlamydial infections. Screening women who were either age ⩽19 years or in their second or third trimester at their first clinic visit would have required testing 62% of the women and would have identified 84% of the infections.

The two women with gonorrhoea were 17 and 22 years old, both were co-infected with chlamydia, and neither was HIV seropositive. The five women with serological evidence of syphilis were between 24 and 35 years old (mean 30 years); one was co-infected with both chlamydia and HIV.

Discussion

In this study of STDs among pregnant women in Thailand in 1996 we found a lower rate of gonorrhoea and syphilis than is found in most other studies of pregnant women, including prior studies in Thailand. The chlamydial infection rate was also lower than in prior Thai reports, which used less sensitive assays and presumably detected fewer infections than might have been found with PCR testing. The low rates of bacterial STDs among pregnant women in Thailand in 1996 suggest that infection rates are declining in the general population as well as among the high risk groups which are the focus of the government's STD surveillance and condom promotion campaign.[14 15 19]

GONORRHOEA

We found a prevalence of gonorrhoea of 0.2%. In a study done during 1993 and 1994 using a nucleic acid hybridisation test (Gen-Probe PACE 2 System, San Diego, CA, USA) at the same two Bangkok hospitals, the prevalence of gonorrhoea among pregnant women was 1.4%.[20] In a much earlier Bangkok report, *N gonorrhoeae* was detected by cervical culture in 10.6% of pregnant women in 1974.[21] In addition to changes in sexual risk behaviour, availability of oral, single dose fluoroquinolone therapy and the mass treatment of sex workers with ciprofloxacin in the early 1990s[22] may have contributed to the low rates of gonococcal infection in the present study. In most other studies of pregnant women, most of which are also from developing countries or are of high risk populations in developed countries, the prevalence is between 1% and 5%.[1 23–25]

CHLAMYDIAL INFECTION

In our study the prevalence of chlamydial infection was 5.7%, while in the 1993–4 study in Bangkok using nucleic acid hybridisation the prevalence was 9.2%.[20] In another report from Bangkok done in 1988 using culture, the prevalence was 10.0%.[26] In most other studies of pregnant women the detected prevalence is between 2% and 10%, but is 20% or higher in some populations.[1 24–31] These other studies have used techniques that are less sensitive than the PCR assay (92–97% sensitive)[3 6 8] such as culture (70–85% sensitive in laboratories with experienced staff), nucleic acid hybridisation (85% sensitive), or antigen detection methods (EIA 73–83% sensitive, DFA 80–90% sensitive).[8]

SYPHILIS

Only 0.5% of the women in this study had serological evidence of syphilis and none had a VDRL or RPR titre greater than 1:4, so some of these were likely to have been treated, serofast cases or biological false positive cases rather than active infections. Other data suggest that syphilis rates among pregnant women in Thailand were higher in the past. At the study hospital in Chiang Rai, the VDRL reactivity rate among pregnant women ranged from 2.1% to 3.2% from 1990 to 1994, was 1.9% in 1995 (Chiang Rai Hospital, unpublished data), and declined to 1.0% in our study in the second half of 1996. At Rajavithi Hospital, VDRL rates declined only slightly, from 1.4% in 1992 to 1.1% overall in 1996 (Rajavithi Hospital, unpublished data), while at another large hospital in Bangkok (not one of the study hospitals) the prevalence of syphilis (positive non-treponemal and confirmatory treponemal tests) among pregnant women declined from 3.0% in 1982 and 2.1% in 1985, to 1.4% in 1992.[19] In other reports of syphilis in pregnant women, many of which use a more specific definition of syphilis such as a higher test titre, the prevalence ranges from less than 1% in some low risk populations to over 10% in some developing countries.[1 24 25 32]

URINE SAMPLE PCR TESTING LIMITATIONS

We found that 20% of the women with *C trachomatis* and both of the women with *N gonorrhoeae* had negative follow up tests (performed before treatment was initiated in the clinic). The eight *C trachomatis* PCR negative follow up specimens were also negative by LCR, suggesting that these were true negative results. The negative follow up results may represent a limitation of nucleic acid amplification testing using female urine specimens, such as a low copy number of target DNA; or they may be due to either treatment with antimicrobials from outside the study clinic or to spontaneous clearance of the organism in the interval between the two tests. Additional research is needed to validate the use of urine specimens and the Amplicor CT/NG PCR assay for pregnant women among whom anatomical or hormonal changes may affect test performance.

SENTINEL SURVEILLANCE OF PREGNANT WOMEN

While pregnant women seeking antenatal care are more representative of the general population of women than are commercial sex workers or STD clinic patients, there are some biases present with this sentinel population. Pregnant women may be more likely than women in the general population to have an STD because they are younger, and they are, by definition, sexually active and less likely to be using barrier contraception methods. On the other hand, pregnant women may be less likely to have an STD because infertility is a sequela of STDs. Acute changes in sexual risk

or healthcare seeking behaviours during pregnancy could also affect the rates of acute bacterial STDs. Additional research is needed to define more fully the relation of STD rates among pregnant women to rates among women in the general population.

We conclude that testing of pregnant women using easily collected urine specimens and a sensitive nucleic acid amplification assay is a feasible method of sentinel surveillance for chlamydial infection and gonorrhoea. The low rates of bacterial STDs among pregnant women in Thailand in 1996 supplement government STD case report surveillance data[19] suggesting that the campaign to promote condom use during commercial sex has had a broad impact.

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