THE PREVALENCE OF *CHLAMYDIA TRACHOMATIS* INFECTION IN RURAL THAI WOMEN

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Abstract. A cross sectional study was designed to investigate the prevalence of *Chlamydia trachomatis* among different groups of rural women in the northeast Thailand. The presence of chlamydial antigens in endocervical swabs was detected by ELISA. The prevalences of *Chlamydia trachomatis* were 6.8% (31/485), 5.2% (24/466) and 6.7% (12/179) in women attending antenatal, postpartum and family planning clinics respectively. The average prevalences of *C. trachomatis* among hospital-based and community-based women were 6.1% (67/1,103) and 3.6% (15/411) respectively. In addition, the prevalences of some pathogens including *Candida albicans, Trichomonas vaginalis, Treponema pallidum* and *Neisseria gonorrhoea* among hospital-based and community-based women were 14.2, 2.8, 0.7, 0.2 and 10.9, 5.1, 2.7, 0.0% respectively. It was concluded that *C. trachomatis* was a problem of woman's reproductive health.

INTRODUCTION

So far, there are no standard methods for surveillance of *Chlamydia trachomatis* in public health and medical services of Thailand. Therefore in the past ten years, data regarding *C. trachomatis* could be obtained mainly from big central and university hospitals. These were from selective cases of patients attending the hospitals (Niamsanit *et al,* 1988; Niruthisard *et al,* 1991; Rugpao *et al,* 1993). As socioeconomic changes occured in the past decade, rural population became high risk and exposed to many types of sexually-transmitted diseases including chlamydial diseases. Still chlamydial prevalence has not yet been reported conclusively among rural women. This might be due to two factors : i) there were no simple and economic methods for diagnosis of *C. trachomatis*, and ii) nobody was really interested in the study of chlamydial diseases in the remote areas. In fact, chlamydial diseases are now common. *C. trachomatis* can cause a wide range of human diseases from eye to sexual and gynecological complications (Rugpao *et al,* 1993). Repeated infections could lead to blindness and infertility (Ridgway and Robinson, 1991; Beatty *et al,* 1994). It was also reported that *C. trachomatis* was accounted for 44-73% of acute pelvic inflammatory diseases (PID) leading to infertility due to tubal occlusion (Czerwenka *et al,* 1994; Heinonen and Miettinen, 1994; Videla *et al,* 1994). The aim of this report was to reveal the prevalence of *C. trachomatis* among rural women with normal health in the northeast Thailand.

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MATERIALS AND METHODS

Subjects

Two groups of subjects were recruited. **Group 1** included women who attended antenatal (pregnant women), postpartum, family planning and general (women attending clinic for general health checking up) clinics, Chum Phae District Hospital, Khon Kaen Province. It was 80 km away from Khon Kaen City. This group of subject, so called "Hospital-based Population" (HBP), constituted of 845 women of 15-54 years old. In addition, 258 pregnant women were recruited from antenatal clinic of Srinagarinda University Hospital, Khon Kaen University. All types of clinical specimens from this group were collected at the clinics of the hospitals. **Group 2** constituted of 411 women of the same age as group 1. They were in four villages in Nam Phong District, Khon Kaen Province. The district was 60 km away from Khon Kaen City. The group members, so called "Community-based Population" (CBP), were volunteers for gynecological and sexual disease investigation. Clinical specimens from the second group were collected at the mobile clinic sited in the villages.

Specimen collection

Endocervical swab : Specimen collection and storage were carried out according to the instruction manual of the Microtrak II Chlamydia EIA kit (Syva Company, San Jose, USA). Briefly, the mucous plug around the exocervix was cleaned up by wipping with a cotton swab. Another large swab was inserted into endocervical canal until most of the tip was not visible. The swab was rotated for 5 seconds inside endocervical canal and withdrawn with out touching any vaginal surface. It was placed in a transport medium tube of the diagnostic reagent kit. These endocervical swabs were stored at 2-8°C and tested with in 7 days.

Vaginal swab : Two vaginal swabs were prepared to collect samples from posterior fornix. One swab was dipped into a saline solution tube. The sample was for wet preparation for microscopic examination. The second swab was directly smear onto the surface of chocolate and blood agar plates, one each. The preparation was for isolation and identification of *Neisseria gonorrhoea.*

Blood : A venous blood sample (5ml) was collected from each woman. After blood clotting, sera were stored at-20 °C until use.

Laboratory tests

ELISA : A Microtrak II Chlamydia EIA reagent kit (SYVA, USA) was applied to detect chlamydial antigens in endocervical samples. The assay was carried out according to the instruction manual of the reagent kit, briefly as followed. All clinical samples and controls were treated with 1 ml specimen treatment solution and heated in a pre-heated water container at 95-100 °C for 15 minutes and cooled down at room temperature for 10 minutes. After standing the reagent kit at room temperature for 15 minutes, an aliquot of 100 µl of rabbit anti-chlamydia immunoglobulin was pipetted into each assigned well. Immediately, it was followed by 100 µl of each sample and controls into the wells. The microplate was standed in a 37 °C moisture chamber for 90 minutes. At the end of incubation, the plate was washed 5 times and followed by incubation with peroxidase-conjugated goat anti-rabbit immunoglobulins 100 µl per well for 30 minutes at 37 °C. After washing 5 times the plate was further incubated with 100 µl per well of substrate solution at 37 °C for 30 minutes. The reaction of the mixture was stopped by 1N H_2SO_4 100 µl / well and the OD values were measured at 492 nm.

The presence or absence of chlamydial antigens was determined by comparing OD values of samples with the cut off OD value which was determined according to the instruction manual (*ie* cut off OD = mean negative control OD + 0.200).

RESULTS

The prevalence of *Chlamydia trachomatis*

A number of endocervical swabs was collected from groups of women attending antenatal, postpartum, family planning and general clinics at Chum Phae District Hospital. In addition to this, 258 samples were collected from antenatal clinic of Srinagarinda University Hospital. Samples were tested for the presence of *C. trachomatis* antigens by ELISA. Results are presented in Table 1. The prevalence of *C. trachomatis* among pregnant women was 6.8% (31/458). Similar rates of chlamydial infection were found among pregnant women in the two hospitals, *ie*, 18 in 258 (7.0%) in Srinagarinda University Hospital and 13 in 200 (6.5%) in Chum Phae District Hospital. The prevalence rates found in postpartum, family planning and general clinics were 5.2% (24/466) and 6.7% (12/179) respectively. The overall result of chlamydial prevalence among rural women was 6.1% (67/1,103).

Table 2 demonstrates the prevalence of *C. trachomatis* in two populations of women, the Chum Phae District Hospital (HBP) and the 4 villages in Nam Phong district (CBP). The prevalences in the HBP and CBP were 5.8% (49/845) and 3.6% (15/411) respectively. The difference in the prevalence rates was significant ($p < 0.05$). Chlamydial infection rates found in different age groups are shown in Table3. The rate of infection tended to increase slightly with ages (3.0 to 15.6%) among women attending hospital while it was rather similar in different age groups of women whose samples were collected at village sites.

The prevalences of other STD pathogens

In addition to the chlamydial study, vaginal swab and blood samples were collected and subjected to investigation for *Candida albicans, Trichomonas vaginalis, Neisseria gonorrhoea* and *Treponema pallidum.* The percentages found are demonstrated

in Table 4. *C.albicans* was found the highest prevalence rate, 14.2% in HBP and 10.9% in CBP. The average percents of positive tests of *C.albicans, T.vaginalis, T.pallidum* and *N.gonorrhoeae* were 12.5, 4.0, 1.8 and 0.1 respectively. The percent positive test of *T.vaginalis* in samples collected from villages (5.1%) was higher than that from Chum Phae District Hospital (2.8%). Similarly, the seroprevalence rates of *T.pallidum* among village samples (2.7%) was significantly higher than that among hospital samples (0.7%, $p < 0.05$). Furthur more, *N. gonorrhoea* was found in only one sample obtained from Chum Phae District Hospital and none was found in 586 samples from villages.

Table 1

The prevalence of *Chlamydia trachomatis* among rural women visiting different clinics at Chum Phae District Hospital, Khon Kaen.

Clinics	No. tested	: No.positivity (%)
Antenatal (Pregnant women)	458*	: 31 (6.8)
Postpartum	466	: 24 (5.2)
Family planning and general clinics	179	: 12 (6.7)
Total	1,103	: 67 (6.1)

* Of 458 samples, 258 were obtained from Srinagarinda Hospital, Faculty of Medicine, Khon Kaen University and 18 samples (7.0%) were positive.

Table 2

The comparison of *Chlamydia trachomatis* prevalence between the hospital-based and community-based populations.

Populations	No. tested	: No. positivity (%)
a) Hospital-based :		
Pregnant women		
Chum Phae Community Hospital	200	: 13 (6.5)
Srinagarinda (Khon Kaen University) Hospital	258	: 18 (7.0)
Non-pregnant women	645	: 36 (5.6)
Total	1,103	: 37 (6.1)
b) Community-based : Total (all non-pregnant women)	411	: 15 (3.6)

Table 3

Infection rates of *Chlamydia trachomatis* found among different age groups of rural women.

Age groups (years)	No. tested : No. positivity (%)		
	Hospital-based women	Community-based women	Total
15-19	133 : 4 (3.0)	8 : 0 (0.0)	141 : 4 (2.8)
20-29	541 : 31 (5.7)	95 : 5 (5.3)	636 : 36 (5.7)
30-39	139 : 9 (6.5)	118 : 6 (5.1)	257 : 15 (5.8)
40-54	32 : 5 (15.6)	88 : 4 (4.6)	120 : 9 (7.5)
Total	845 : 49 (5.8)	411 : 15 (3.6)	1,256 : 64 (5.1)

Table 4

The prevalence of other STD pathogens found among rural women in the northeast Thailand.

STD pathogens	No. tested : No. positivity (%)		
	Chum Phae (Hospital-based) women	Nam Phong (Community-based) women	Total
Candida albicans	530 : 75 (14.2)	586 : 64 (10.9)	1,116 : 139 (12.5)
Trichomonas vaginalis	530 : 15 (2.8)	586 : 30 (5.1)	1,116 : 45 (4.0)
Treponema pallidum	530 : 4 (0.7)	586 : 16 (2.7)	1,116 : 20 (1.8)
Neisseria gonorrhoea	530 : 1 (0.2)	586 : 0 (0.0)	1,116 : 1 (0.1)

DISCUSSION

Many methods were applied to diagnosis of *Chlamydia trachomatis* infections. These included cell culture, DNA probe, PCR, direct immunofluorescence and ELISA. In terms of its sensitivity and specificity, the cell culture method seemed to be the most appropriate assay for the well setting laboratories. However with a large number of clinical samples to be screened, the method might have limitation. ELISA (SYVA) had been applied to screen 1,256 samples of endocervical swab for the presence of *C.trachomatis* antigens since it had been shown to have satisfactory sensitivity and specificity (Olsen *et al,* 1995).

We reported about 6.8% of pregnant women in rural communities possessed chlamydial antigens in their genital tract. The finding was similar to that described by Koroku *et al* (1994). They reported 5.6% detection rate of *C.trachomatis* among pregnant, married women and 15.2% among pregnant, unmarried women who underwent artificial abortions in Hokkaido, Japan. This might imply that women with promiscuous sexual behaviors would have high risk and high infection rate of *C. trachomatis.* In rural societies of Thailand, it is unusual for women to practice such behaviors. The underlying prevalence of 6.8% might result from their husbands who left home seasonally for jobs in big cities. Similar prevalence of *C.trachomatis* was found among the postpartum group (5.2%) and women visiting family planning and general clinics for health checking up (6.7%, Table 1.) In other societies, for example the hispanic Mexican-Americans *C.trachomatis* prevalence was reported at 10.1% (Compos-Outcalt and Ryan, 1995). Rappai *et al* (1995) had shown a very unusually high prevalence of 38.8%. It was even much higher than that found in the high risk groups, usually ranging from 8 to 27% (Hudson *et at,* 1994; Rugpao *et al,* 1993; Wathne *et al,* 1994). Ten years ago, Niamsanit *et al,* (1988) reported 24% positive rate of *C.trachomatis* among selective pregnant women age 20-24 attending antenatal clinics in Bangkok. This study showed about 5-6% positive rate among pregnant women, age 20-29, in rural and hospital communities (Table 3). It is likely that the decrease of *C. trachomatis* prevalence was due to the successfulness of national campaign program against HIV/AIDS during the past decade. Unlike other findings that *C.trachomatis* tended to have high prevalence in younger age group (*ie* 15-25) this study indicated a low prevalence of *C. trachomatis* in younger age group 15-19 (2.8%) and similar prevalence in all age groups (Table3) excepted that the HBP age group 40-54, possessed 15.6% of *C.trachomatis* prevalence. This could be accounted by i) too small sample size and ii) the high prevalence rate of *C. trachomatis* reported in the past 10 years still persisted in this group of women while a younger generation practiced a better self-protection behaviors against sexually-transmitted diseases (STDs) and HIV/AIDS. However it was observed, in a selective group of women attending STD clinic, that younger age group (15-24) had higher prevalence of *C.trachomatis* (10.8%) than older age groups (Tswana *et al,* 1995). The prevalences of *C. trachomatis* among age groups therefore depened on nature of the population, *ie,* profession, sexual behaviors leading to high risk of infection of STDs. Results in Table 3 support the concept that different groups of population possess different rates of chlamydial infection. The rate was significantly high in the HBP group (6.1%). This might be explained by the nature of specific and selective ages and risk facfors possessed by women

attending hospital for specific purposes. While the community-based study covered a broad range of age and risk groups.

In many low-income societies, the prevalence of STD tended to be highly endemic (Compos-Outcalt and Ryan, 1995; Hudson *et al,* 1994; Tswana *et al,* 1995). This report showed that *Candida albicans* was the most frequently found (12.5%). Its prevalence in the HBP group was also higher than that in the CBP group. *C.albicans* infection occurred in association with the application of contraceptive methods and non-hygienic behaviors. The infection is usually harmless but it can cause diseases in certain circumtances (Desmond *et al,* 1971; Lazar, 1971). In contrast *Trichomonas vaginalis* was found higher prevalence in the CBP group (5.1%) compared to that in the HBP (2.8%). This might reflect the practice of non-hygienic behaviors among rural peoples, particularly in this study the sexual behaviors and the accessibilities to health and medical services which were later limited in rural areas compared to the town and urban ones. It was interesting that the seroprevalence of syphilis among HBP women (0.7%) was clearly lower than that found in the CBP women (2.7%). When the age of these two groups of women were analysed, it was indicated that about 20% (171/845) of the HBP and 50% (206/411) of the CBP were more than 30 years old (Table 3). This suggested that older women in the communities possessed high seroprevalence of syphilis. However, this could be resulted from the past infection of *Treponema pertenue*. The *T. pertenue*, causing yaws had been reported in many areas of the northeast among older peoples in the past. In our recent seroprevalence survey, it was demonstrated that seroprevalence against syphilis increased with age up to 60-70 years of age (data not shown). This was accounted by the past history of *T.pertenue* infection. The low prevalences of *T.pallidum* (0.7% in younger HBP women) and *N.gonorrhoeae* (0.1-0.2% in both groups) might be resulted from an effectiveness of the national campaign program against HIV/AIDS.

In conclusion, even though many types of STD prevalence were decreasing, the *C. trachomatis* diseases still could be found. The *C. trachomatis* screening test should be included in the prenatal check up since the organism still existed with relatively high prevalence and caused many serious complications in women and infants.

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