CHAPTER IV

RESULTS

Characteristic of C. trachomatis in McCoy Cell by Iodine Staining

Specimens were inoculated into McCoy cell treated with cycloheximide. The formation of iodine-staining intracytoplasmic inclusions in inoculated cell cultures was considered to show that C. trachomatis was present in a specimen. Because chlamydiae, like viruses, are obligate intracellular parasites, patients from whom C. trachomatis was isolated were regarded as infected.

C. trachomatis was characterized by compact round or oval glycogen-containing inclusions within the cytoplasm. The inclusions were dark-brown or red-brown in color. They were easily detectable because they stained much more deeply than the McCoy cells. The pictures of C. trachomatis inclusions were in Figure 4 and Figure 5.

At 72 hr there is much greater variation in size of inclusions and also a greater variation in depht of staining. At this interval some large inclusions appear quite pale, and in such inclusions the granular nature of the iodine stained material is espectially evident.

Characteristic of Chlamydial Seropositive by MIF Test

The MIF test measures specific antibodies to antigenic determinants present in the cell walls of the elementary body particles. The MIF test may detect both IgM and IgG.

The positive results mean the presence of sharply demarcated, brightly fluorescing particle when compared to an appropiate control. The endpoint was considered to be the highest dilution of serum

recorded positive. The pictures of negative control, positive control and positive high titer of MIF test for C. trachomatis were shown in Figure 6, Figure 7 and Figure 8, respectively.

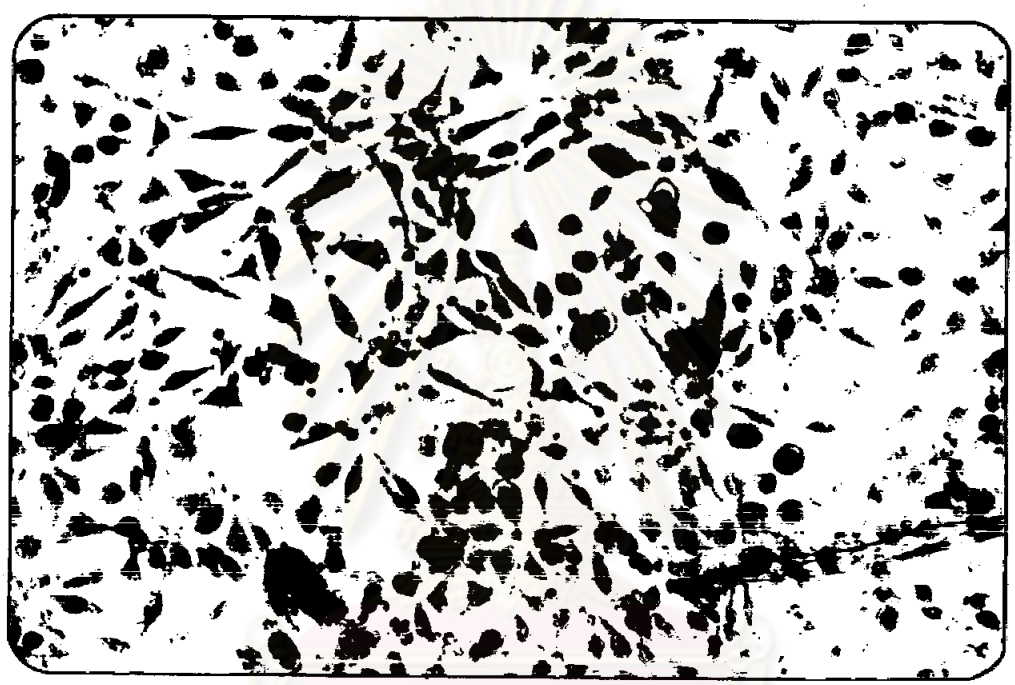

Figure 4 C. trachomatis inclusions in McCoy cells with iodine staining (10x)

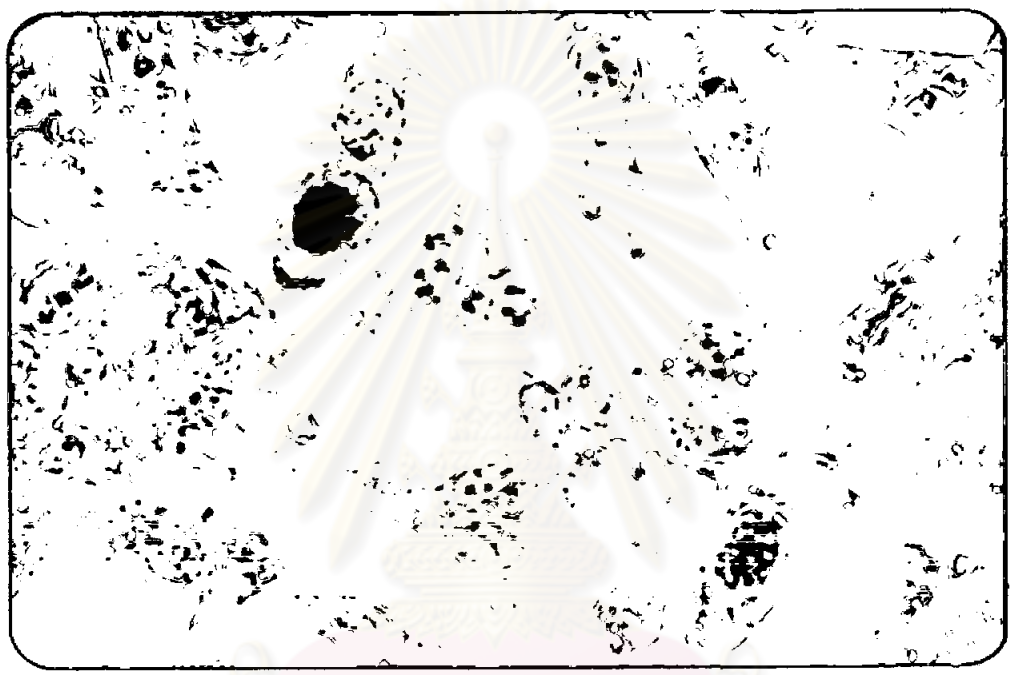

Figure 5 C. trachomatis inclusions in McCoy cells with iodine staining (40x)

Figure 6 Negative control of MIF test for C. trachomatis (40x)

Figure 7 Positive control of MIF test for C. trachomatis (40x)

Figure 8 Positive high titer of MIF test for C. trachomatis (40x)

Prevalence of Infection

During the period of this study, endocervical cultures for C. trachomatis were performed on 200 of first trimester pregnant women. C. trachomatis was detected in 20 women, or 10% of the group.

From 140 of the 200 women, whose specimens were obtained in paired, the first collected during the first trimester and the second during the third trimester. C. trachomatis was isolated from 15 (10.7%) of these 140 women in the first trimester and from 18 (12.8%) in the third trimester. This difference in rates of isolation was not statiscally significant ($p > 0.05$, χ^2 test, df=1). In addition, in the first and/or in the third trimester of pregnancy, C. trachomatis were found 21 (15%). Although no antibiotic therapy was given to positive culture of the first trimester pregnant women, three women with positive culture in the first trimester become to culture negative in the third trimester. Positive or negative culture of these 140 women during the first and third trimester is recorded in Table 9.

Table 9 Frequency of C. trachomatis isolation during the first and third trimester of pregnancy from 140 cases

C. trachomatis isolation in first / third trimester	No.	%
negative / negative	119	85.0
positive / positive	12	8.6
negative / positive	6	4.3
positive / negative	3	2.1
total No. with isolation positive during first trimester	15	10.7
total No. with isolation positive during third trimester	18	12.8

Total No. tested = 140

Serology

In this study, 48 sera of normal women who never had sexual contact were included to find the lowest important titer of IgG. The result of which was shown in Table 10.

Table 10 IgG antibodies titer of 48 normal women sera

Titer	No. positive sera
1:8	48
1:16	0

The result shows that IgG antibody titer of 1:16 was an important titer in detecting antichlamydial IgG antibody.

1. Antichlamydial IgG and C. trachomatis Isolation

The incidence of high level antibody (IgG titer ≥ 1:64) associated with positive isolation was found in 14/18 (77.7%) and in 8 (7.5%) out of 106 of those with isolation negative results. Thus, there was no significant difference in the number of patients with positive results to combined serodiagnostic test ($p > 0.05$, χ^2 test, df=1) (Table 11).

Table 11 Relationship of antichlamydial IgG to genital serotype with the presence of C. trachomatis in cell culture

Titer of IgG	No. of C. trachomatis isolation positive in			No. of C. trachomatis isolation negative in first and third trimester
	first trimester	third trimester	first and/or third trimester	
< 1:16	1	2	3	91
1:16	2	1	2	1
1:32	0	1	1	6
1:64	3	6	6	4
1:128	4	4	4	2
1:256	4	4	4	0
1:512	0	0	0	2

Total No. tested = 126

Geometric Mean Titer = 1:57

2. Distribution of Antibodies to Chlamydial Serotypes

The distribution of antibodies to chlamydial serotypes in the 126 women examined was shown in Table 12 . Antibodies to C. trachomatis in pool I (A to C serotypes) were found in 1 (0.8%); pool II (D to K serotypes) in 18 (14.3%); pool III (L_1 to L_3 serotypes) in 1 (0.8%); pool II,III in 5 (4.0%); and pool I,II,III in 7 (5.5%) of pregnant women attending antenatal clinic. Thus, there were 30 (23.8%) women had antichlamydial antibody to C. trachomatis serotype D to K which are genital serotypes.

Table 12 Distribution of antibodies to chlamydial serotypes in the pregnant women

Serotype of C. trachomatis	Women with antibodies to C. trachomatis	
	No.	%
pool I[a]	1	0.8
pool II[b]	18	14.3
pool III[c]	1	0.8
pool II,III	5	4.0
pool I,II,III	7	5.5
Total No. tested = 126		

a = A to C serotypes

b = D to K serotypes

c = L_1 to L_3 serotypes

3. prevalence of Antibodies to C. trachomatis Serotypes D to K

The prevalence of IgG and IgM antibodies specific for C. trachomatis serotypes D to K was shown in Table 13. Antibodies suggesting active chlamydial disease (IgG Ab titer ≥ 1:64) were found in 21 (16.7%) of these group. IgM antibody was not found.

Table 13 Prevalence of antibodies to C. trachomatis serotypes D to K in 126 pregnant women

	Women with antibodies to C. trachomatis serotype D to K
No. (%) with IgG Ab titer=1:16-1:32	9 (7.1)
No. (%) with IgG Ab titer ≥ 1:64	21 (16.7)
No. (%) with IgM Ab	0 (0)
Total No. (%) with chlamydial Ab	30 (23.8)

Symptoms of Lower Genital Tract Infection

Relationship between endocervical C. trachomatis infection and symptoms of lower genital tract infection either on the first or third trimester of pregnancy was shown in Table 14. Chlamydial infection was not associated with any particular symptoms, except vaginal bleeding. 3/20 (15.0%) women whose chlamydial culture positive and 5/180 (2.8%) women whose chlamydial culture negative had vaginal bleeding in the first trimester ($p < 0.05$, χ^2 test).

In the third trimester of pregnancy, only one (5.5%) woman had vaginal bleeding,when speculum was inserted, and her chlamydial culture was positive. No one (0%) in chlamydial culture negative group had vaginal bleeding ($p > 0.05$, χ^2 test).

Table 14 Relationship of symptoms of lower genital tract infection with isolation of C. trachomatis in the first and third trimester of pregnancy

Symptoms (patients' report)	C. trachomatis positive		C. trachomatis negative		P (χ^2 test)
	No	%	No	%	
first trimester	20		180		
vaginal discharge	11	55.0	85	47.2	> 0.05
vaginal itching	3	15.0	40	22.2	> 0.05
dysurea	2	10.0	20	11.1	> 0.05
frequent urination	18	90.0	147	81.7	> 0.05
vaginal bleeding	3	15.0	5	2.8	< 0.05
third trimester	18		122		
vaginal discharge	9	50.0	48	39.3	> 0.05
vaginal itching	3	16.7	23	18.8	> 0.05
dysurea	0	0	8	6.5	> 0.05
frequent urination	18	100.0	113	92.6	> 0.05

History of Sexually Transmitted Disease (STD)

The history of STD was associated to a statistically significant with antichlamydial antibody ($p < 0.05$, χ^2 test). In the women examined 7/12 (58.3%) had history of STD, while 26/114 (22.8%) had no history of STD. Alternatively, the history of STD was not associated with chlamydial cultures ($p > 0.05$, χ^2 test) as shown in Table 15.

Table 15 The association of history of STD with culture or serology of *C. trachomatis*

Subjuct	*C. trachomatis* culture positive		IgG titer ≥ 1:16	
	No.	%	No.	%
with history of STD	3/15	20.0	7/12	58.3
without history of STD	18/123	14.6	26/114	22.8
	$p > 0.05$ χ^2 test		$p < 0.05$ χ^2 test	

Age Distribution

The age distribution of the chlamydial infected women were significant higher among younger than among older pregnant women. In 140 women, 14(23.7%) of those aged 20 to 24 years were chlamydia positive , whereas only 7 (8.6%) of the women aged 25 to 30 years shed chlamydia (Table 16). On the other hand, the association of the subject's age with antichlamydial antibody to C. trachomatis was not statistically significant ($p > 0.05$, χ^2 test) as shown in Table 16. 17 (30.9%) and 15 (21.1%) of the women aged 20 to 24 years and 25 to 30 years respectively, had antichlamydial antibody to C. trachomatis.

Table 16 Relationship of age group with isolation or serology of C. trachomatis

Subjects' age group	C. trachomatis culture positive		IgG titer ≥ 1:16	
	No.	%	No.	%
20-24 years	14/59	23.7	17/55	30.9
25-30 years	7/81	8.6	15/71	21.1
	$p < 0.05$ χ^2 test		$p > 0.05$ χ^2 test	

Parity

The prevalence of C. trachomatis isolation in primigravida, 17(18.3%) of 93 women, was higher than in multigravida, 4(8.5%) of 47 women. However, the difference was not statistically significant ($p > 0.05$, χ^2 test).

Also the proportion of the women with serologic evidence of chlamydial infection (titer $\geq$ 1 : 16) was not influenced by parity (Table 17).

Table 17 The association of parity with isolation or serology of C. trachomatis

Parity	C. trachomatis culture positive		IgG titer $\geq$ 1 : 16	
	No.	%	No.	%
primigravida	17/93	18.3	20/83	24.1
multigravida	4/47	8.5	12/43	27.9
	$P > 0.05$ χ^2 test		$P > 0.05$ χ^2 test	

Schooling

The association of chlamydial isolation with their schooling of ⩽ 7 years, 8-12 years and > 12 years (college) were 13/81 (18%), 8/46 (21.1%) and 0/13 (0%), resprctively. Also, the association of chlamydial serology (titer ⩾ 1:16) with their schooling were 18/74 (24.3%), 13/40 (32.5%) and 1/12 (8.3%), respectively (Table 18). Nevertheless, both of the difference of the associations were not statistically significant ($p > 0.05$, χ^2 test).

Table 18 The association of schooling with isolation or serology of *C. trachomatis*

Schooling	*C. trachomatis* culture positive		IgG titer ⩾ 1:16	
	No.	%	No.	%
⩽ 7 years	13/81	18.0	18/74	24.3
8-12 years	8/46	21.1	13/40	32.5
> 12 years (college)	0/13	0	1/12	8.3
	$p > 0.05$ χ^2 test		$p > 0.05$ χ^2 test	

Antimicrobial Susceptibility

Of the 30 strains of C. trachomatis tested, they were highly susceptibility to the antibiotics tested. The range of antibiotics susceptibility were quite narrow. The percentage of strains inhibited by any antimicrobial agents was shown in Table 19, and the cumulative percentage of strains inhibited by any antimicrobial agents was shown in Figure 9.

Table 19 Percentage of 30 strains of C. trachomatis inhibited by any antimicrobial agents

Antimicrobial agents	Percentage of strains inhibited by indicated concentration (µg/ml)		
	0.06	0.12	0.25
Tetracycline	43.3	53.4	3.3
Erythromycin	6.7	86.6	6.7
RU 28965	66.7	33.3	

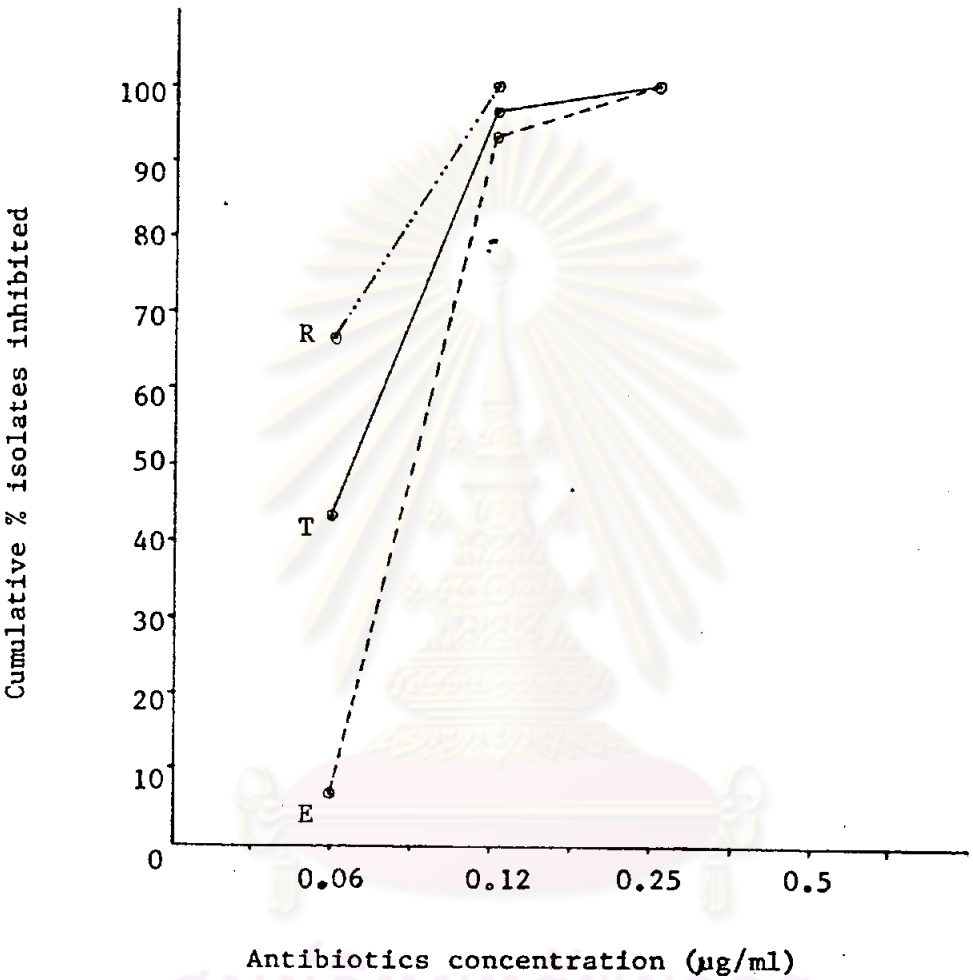

Figure 9 Cumulative percentage of inhibition of isolates by tetracycline (T), erythromycin (E) and RU 28965 (R).

$\underline{MIC_{50}}$ and $\underline{MIC_{90}}$

The results of drug susceptibility tests were summarized in Table 20. All the antimicrobial agents tested were active against the C. trachomatis strains tested. The MIC_{50} and MIC_{90} of tetracycline, erythromycin and RU 28965 were 0.07, 0.11; 0.09, 0.12; and 0.06, 0.10 µg/ml, repectively.

Table 20 The MIC_{50} and MIC_{90} of antimicrobial agents against 30 strains of C. trachomatis

Antimicrobial agent	Range (µg/ml)	Inhibitory concentration (µg/ml)	
		MIC_{50}	MIC_{90}
tetracycline	0.06-0.25	0.07	0.11
erythromycin	0.06-0.25	0.09	0.12
RU 28965	0.06-0.12	0.06	0.10