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SUMMARY

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Running title: *Chlamydia trachomatis* in Guadeloupe

KEY MESSAGES

The prevalence of *Chlamydia trachomatis* urogenital infection was 16.9% in men and 9.8% in women in 2000 in Guadeloupe, French West Indies.

The distribution of *Chlamydia trachomatis* genotypes responsible for urogenital infections in Guadeloupe, French West Indies, differed from that in mainland France.

The SERO-CT assay was unable to detect antibodies in the serum samples of patients infected with *Chlamydia trachomatis* genotype Ia strains.

STATEMENT

COMPETING INTEREST

None declared.

ABSTRACT

Objectives: To determine the prevalence and genotypes of *Chlamydia trachomatis* urogenital infection in Guadeloupe, French West Indies. To compare *C. trachomatis* direct detection to serological testing.

Methods: From March to November 2000, 971 consecutive patients (888 women and 83 men) who had been referred to clinical laboratory of the *Institut Pasteur de la Guadeloupe* for routine testing for genital infection, were recruited. Samples were subjected to a nucleic acid amplification assay (AMP CT, Gen-Probe). Genotypes were determined by *omp1* PCR-RFLP analysis. Serological testing was carried out with the commercially available peptide-based ELISA assay (SERO-CT IgG/IgA, Savyon/BMD).

Results: Positive AMP CT test results were obtained for 102 (10.5%) of the 971 samples. The prevalence of infection was 16.9% in men and 9.8% in women. The most common genotypes were E (34.3%), F (23.9%), Da (13.4%), I (9%), and Ia (7.5%). No relationship was found between genogroups and age, sex or clinical symptoms. With AMP CT used as a reference, the sensitivity, specificity, positive, and negative predictive values of SERO-CT were 81.1%, 56%, 34.5% and 91.2 %, respectively, for IgG and 55.4%, 76.8%, 59.4 %, and 85.8 %, respectively, for IgA. IgG seroprevalence rates were very low (1/5, 20%) in patients infected with genotype Ia strains.

Conclusions: The prevalence found in Guadeloupe did not differ not significantly from that which was found in mainland France. The genotypes Da, F, I , and Ia were more prevalent in Guadeloupe; however, the SERO-CT assay was unable to detect serum antibodies in 80% of the patients infected with genotype Ia strains.

INTRODUCTION

Chlamydia trachomatis is considered to be the most common bacterial cause of sexually transmitted infections (STIs) worldwide. It is currently classified into 19 different serovars on the basis of immunogenic epitope analysis of the major outer membrane protein (MOMP). Serovars D to K, and related variants, Da, Ia, and Ga, are generally found to be associated with urogenital infections. [1,2] Over the last two decades, genotyping based on the *omp1* gene, encoding MOMP, has become a convenient alternative to the laborious serotyping method. The single-copy *omp1* gene displays extensive sequence variations in four regions (VS1-VS4), interspersed with five regions of conserved sequence. The diversity of the DNA can be analyzed by restriction fragment length (RFLP) analysis and/or by sequencing of the PCR-amplified *omp1* gene. [1-11] Genotyping was originally carried out on cell cultures, but direct genotyping of clinical specimens is now performed by sensitive nested PCR of the *omp1* gene.[12] Phylogenetic analysis of *omp1* subdivides strains into three distinct genogroups : B (serovars B, Ba, D, Da, E, L1 and L2), intermediate (serovars F, G, and Ga), and C (serovars A, C, H, I, Ia, J, K, and L3). [1,2]

In Guadeloupe, French West Indies, there is no data in the literature concerning *C. trachomatis* urogenital infection. In mainland France, commercially available PCR methods have showed that the prevalence of such infection in symptomatic populations was 8-10.2% in women and 16.4% in men (1994-1998). [13, 14] In the Caribbean region, the prevalence ranges from 5.4% to 21% in asymptomatic women from Curaçao, Barbados, and Trinidad, to 55% in STI clinic patients from Jamaica. [15-19] Despite the use of different methods and populations, which made the results difficult to compare, these

studies provide useful data to national health authorities. A piece of valuable information that has not yet been reported in the Caribbean region are the genotypes or serovars of circulating *C. trachomatis* strains.

We report here the first study on *C. trachomatis* urogenital infection in Guadeloupe. We established the prevalence of this infection by carrying out nucleic acid amplification tests (NAATs) on a population of 971 consecutive patients referred for routine testing for urogenital infection, and determined the genotypes by PCR-RFLP. We searched for relationships between genogroups and particular demographic, clinical, serological, and microbiological characteristics of the infected population.

MATERIALS AND METHODS

Study population

Guadeloupe is an overseas French *département* covering 1705 km^2 and located in the Caribbean region. The archipelago had a total population of 422,000 inhabitants (1999 census data).

The selected population consisted of 971 consecutive patients (888 women and 83 men), who were referred to the clinical laboratory of the *Institut Pasteur de la Guadeloupe*, Pointe à Pitre, Guadeloupe, by general practitioners, dermatologists or gynaecologists during the period from March to November 2000 for routine testing for urogenital infection. All the specimens were collected and all assays other than genotyping were carried out in the laboratory.

Data, including demographic characteristics such as age and sex, clinical

symptoms and results of all microbiological investigations were recorded for two subsets of patients. The first subset was comprised of patients with a positive NAAT (n=102). The second subset comprised of those with an available serological test (n=333), was classified as follows: one CT+ group (n=74; median age: 24.6±7.4 years; range: 13-44) comprised of patients, 65 of which were women and 9 of which were men, with AMP CT-positive assays; and one CT- group (n=259; median age: 29.2±9.9 years; range: 14-70) comprised of 238 women and 21 men. Ethical approval was not required under local law for this study due to its retrospective design and use of data obtained through routine testing.

C. trachomatis nucleic acid detection

C. trachomatis direct detection was performed with the Amplified *Chlamydia trachomatis* assay (AMP CT; Gen-Probe, San Diego, USA). This NAAT targets a specific 23S rRNA of *C. trachomatis* in a transcription-mediated amplification and hybridisation protection assay. Male urethral (n=72) and female endocervical (n=867) swabs or first-void urine specimens from men (n=11) or women (n=21) were collected and tested according to the manufacturer's instructions.

C. trachomatis antibody detection

C. trachomatis antibody status was determined on 333 sera from 333 patients with the SERO-CT IgG/IgA test (Savyon/BMD, Marne-La-Vallée, France), according to

the manufacturer's instructions. This microtiter-based ELISA uses a mixture of species-specific peptides from the *C. trachomatis* MOMP for the detection of specific IgG and IgA antibody. Samples with index values (OD sample/OD negative control x 2) ≤ 1.1 were considered negative, whereas those with index values > 1.1 were considered positive.

Genotyping of *C. trachomatis*

Of the 100 AMP CT-positive non-urine specimens, stored in a Gen-Probe transport tube at –70°C, 99 were transported frozen, overnight, to the French National Reference Centre for *C. trachomatis*. Samples were diluted 1/10 with lysis buffer and nested PCR analyses were performed by using primers NLO and NRO in the first step and PCTM3 and SERO2A in the second step, as described previously. [5, 6] The RFLP analysis of nested PCR products was carried out by using *Alu*I, *Hpa*II-*Eco*RI-*Hinf*I, *Cfo*I, *Dde*I, and *Fok*I, as described previously. [5, 6]

Statistical analysis

Univariate comparisons were based on the Pearson's chi-squared statistic or two-tailed Fisher's exact tests for categorical variables, and on Student's t tests or analysis of variance for continuous variables. Analyses were performed with Stat software (Stata 7.0; Stata, College Station, USA). A p value of 0.05 was considered statistically significant.

RESULTS

C. trachomatis prevalence and characteristics of the infected population

Positive results in the NAAT were obtained for 102 of the 971 samples (10.5%). The prevalence of *C. trachomatis* was 16.9% in men (15/83) and 9.8% (87/888) in women. The mean age of infected men and women was 29.4±6.9 years (range 20-44) and 24.4±7.2 years (range 13-44), respectively. Clinical symptoms were present in 89.3% (75/84) of infected women and in 92.9% (13/14) of infected men. The prevalence in women of co-infection with HIV, *Neisseria gonorrhoeae*, *Trichomonas vaginalis* and *Candida albicans* was 5.9% (5/84), 2.3% (2/87), 3.4% (3/87), 19.5% (17/87), and 5.7% (5/87), respectively. Bacterial vaginosis was seen in 58.6% (51/87) of the *C. trachomatis*-infected women. Infected men were co-infected with only one other agent, *N. gonorrhoeae*, in 3 of 14 (21.4%) patients.

Distribution of the *C. trachomatis* genotypes

The *omp1* gene was successfully amplified in 67/99 (67.7%) non-urine positive samples. The distribution of the genotypes is shown in Table 1. The genotypes E (34.3%), F (23.9%), Da (13.4%), I (9%), and Ia (7.5%) were the most common. The genotypes were evenly distributed over the study period (data not shown). Due to the small number of non-E and non-F genotypes, the genotypes were grouped together into

the B, intermediate, and C genogroups for further analysis. [1]

Association of genogroups with demographic characteristics and clinical signs

Table 2 shows the association between demographic characteristics, clinical signs, laboratory data and genogroup. No relationships were found between genogroup and age, sex, clinical symptoms or other associated genital infections other than *N. gonorrhoeae* and the C genogroup (p =0.005). These co-infections (two caused by Ia genotype strains and two by I genotype strains) occurred between July and September.

Relationships between chlamydial infection and serological data

We evaluated the potential usefulness of serological testing, by analysing a subpopulation of 333 patients with an available serological test result with the commercially available peptide-based ELISA, SERO-CT IgG/IgA. A comparison of the serological results with the results of the NAAT is shown in Table 3. The IgG and IgA seroprevalence rates and the mean IgG and IgA ratios in the CT+ group were significantly higher than those in the CT- group.

The sensitivity of SERO-CT for antibody detection (using the NAAT as the reference) was 81.1% for IgG and 55.4% for IgA. The specificity of detection was 56% for IgG and 76.8% for IgA. The specificity was higher (81.5%) for tests for the simultaneous presence of IgG and IgA. Positive predictive values (PPV) were 34.5% for IgG and 59.4% for IgA and negative predictive values (NPV) were 91.2% for IgG and 85.8% for IgA. IgG antibodies were found in 55.6% (35/63) of patients suffering from

lower abdominal pain. They were found in 81.2% (13/16) in such patients in the CT+ group and 46.8% (22/47) of such patients in the CT- group; whereas the occurrence of abdominal pain was similar in the two groups: 22.2% (16/72) and 19.1% (47/246), respectively.

Relationships between genogroup and serological results

Serological results were available for 46 of the 67 patients whose strains were genotyped. Table 4 shows IgA and IgA seroprevalence rates as a function of genogroup. Statistically significant higher IgG seroprevalence rates were found in patients infected with genotypes of the intermediate (100%) and B (92.3%) genogroups than in those infected with genotypes of the C genogroup (40%). Five of the 10 C genogroup strains were of genotype I and the other five were of genotype Ia. IgG was detected in 60% (3/5) of patients infected with strains of genotype I but in only 20% (1/5) of patients infected with strains of genotype Ia.

DISCUSSION

We found the prevalence of *C. trachomatis* urogenital infection to be 10.7% (10% in women and 18.1% in men) in our study population. This prevalence was lower than those observed in symptomatic patients from the Caribbean region but did not differ significantly from that of mainland France.

The failure rate (33%) for determination of genotypes by nested *omp1* PCR on clinical samples in this study was higher than generally reported (< 20%). [10, 20, 21] There may be several reasons for this. It is more difficult to amplify the 1.2 kb *omp1* gene, which is present as a single copy in the bacterium, than the smaller rRNAs present in many thousands of copies. High failure rates are therefore likely for samples with small numbers of micro-organisms or samples containing small number of infected cells. This may account for the higher failure rate in men (50%) than in women (30.2%), due to milder urethral scraping in men. Other hypothesis are the lack of a DNA extraction procedure before PCR (Vanduynhoven *et al*. reported a nested PCR failure rate of 22.9%, which decreased to 4.9% following the use of DNA extraction methods) and the quality of the DNA matrix stored in the Gen-Probe transport medium, which was developed for the stabilisation of RNA molecules. [1]

This study is the first to describe the *C. trachomatis* genotypes circulating in Guadeloupe and in the Caribbean region. The two most prevalent genotypes in Guadeloupe, E and F, have also been reported to be the most prevalent around the world.[9, 11, 20, 22] However, the distribution of some other genotypes differed considerably from that in mainland France: in particular, genotypes Da (13.4% vs 4.5%), F (23.8% vs 13.5%), I (9% vs 3.5%) and Ia (7.4% vs 0.3%) were more prevalent, whereas genotypes

D (5.9% vs 11.2%), G (3% vs 11.8%), H (0% vs 5.1%), and K (0% vs 5.1%) were less prevalent. [23] Genotype Da infections have rarely been described since the first description of this serovar in 1991. [24] No Da genotypes were found among the 507 *omp1* sequences obtained for samples from a large US nationwide study conducted between 1995 and 1997. [9] A genotyping study by PCR-RFLP (*Cfo*I) in 2001 in Cameroon found a prevalence of the Da genotype of 8.6% (3/35), whereas in Senegal in 1996-1997, *omp1* sequencing revealed a prevalence of D/Da genotypes of 19% (4/21). [6, 11] The D/Da strains belong to two distinct phylogenetic lineages. [8] The Da strains from each lineage have a specific single nucleotide polymorphism (SNP) within *omp1* resulting in the loss of a *Cfo*I restriction site and in amino acid substitution alanine to threonine at position 311/312 modifying the epitope recognised by the monoclonal antibodies used for the serotyping of group D strains. [7,8] BLAST analysis can group *omp1* sequences into the appropriate lineage, but it is necessary to identify the SNP encoding the Ala311/312Thr substitution precisely for Da assignment. This may explain why it is more difficult to detect Da variants by *omp1* sequencing than by serotyping or PCR-RFLP, leading to possible underestimation. The PCR-RFLP profiles obtained with the Da and D strains from Guadeloupe were similar to those predicted from the *omp1* sequences of Da/TW-448 (first lineage), and D1 or D2 group (second lineage) strains, respectively.

The serovar variant Ia was described in 1991, following a study of 41 genital isolates isolated worldwide, in which Ia isolates were much more frequent than I isolates (41 vs 9). [24] In the United States, a high prevalence (14%) of genotype Ia was found during the 1995-1997 study. [9] Other studies in the US found that infection with serovar Ia was significantly associated with being black. [25, 26] In a study carried out in 1994 in the Netherlands, male patients from Suriname were significantly more frequently

infected with genotype Ia strains than were men from the Netherlands, and women from Suriname were more frequently infected with genogroup C strains than were women from the Netherlands. [1] In Africa, one study conducted in Senegal, found a genotype Ia prevalence of 4.8% (1/21). [11] In Asia, one studied reported a prevalence of genotype Ia infection of 6.6% in pregnant women in Thailand, whereas another study reported a significant increase in the prevalence of genotype I strains (Ia not differentiated from I) in Japan between the mid 1990s (1/41, 2.1%) and 2003-2005 (10/81, 12.3%). [9, 27] In Guadeloupe, a large proportion of the population is Black and of African descent (about 80 %) and about 15% of the population is "Indian" and of Asian descent. That may explain why genotypes I (9%) and Ia (7.5%) were well represented.

Serological results and direct evidence of infection have not been found to correlate in many reports, even with an ELISA using specific *C. trachomatis* MOMP peptides. [28, 29] This has been partially attributed to the antibody kinetics (i.e., the lag period between being infected and the antibody response and the persistence of antibodies after a resolved infection). Rabenau *et al*. reported a lower specificity (37.3%) and a much lower PPV (< 20%) for IgG detection with SERO-CT than for detection with NAAT. [29] However, a high NPV (96.4%) was found to be useful for identifying patients at high risk in whom *C. trachomatis* infection is unlikely to play a role. SERO-CT was reported to outperform PCR for IgG detection in another study (specificity of 69% and PPV of 50%). [28] We also found that this assay performed well for IgG and IgA detection in comparison with AMP CT. Our results, however, could be affected by patient selection bias as the analyses were done on a subpopulation selected based on the basis on available serology. The requests for serology might indicate a higher risk of *C. trachomatis* infection, in particular upper genital tract infection or recurrent infections.

Indeed, the prevalence of this infection was found to be 21.5% (65/333) in the subpopulation vs 10.7% within the whole population. For optimal serological results, the selection or preparation of the microbial antigen is important. In a follow-up study, the SERO-CT gave false-negative results and the authors suggested that this test might not be suitable for all serovars. [30] We show here that SERO-CT detected no antibody response in 6/10 patients infected with strains of the C genogroup, particularly genotype Ia strains. However, further studies involving more cases are necessary to confirm this finding. The exact composition of the SERO-CT antigen mixture has not been made publicly available, but it seems likely than no Ia-derived peptide was included. Therefore, in seroepidemiological studies using such high-specificity ELISA-based assays, it is essential to establish, before beginning the study, that the ELISAs used are capable of detecting an antibody response in patients infected with the various circulating genotypes.

AUTHOR CONTRIBUTIONS

F-X.W. and B.D.B. designed the study; F-X.W. and S.L.H performed sampling and microbiological analysis; M.C. performed genotyping; C.S. performed statistical analysis; F-X.W. and B.D.B. analysed data; F-X.W., S.L.H, and B.D.B. wrote the manuscript.

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Serovar			Male n	Female n (%)	Total n (%)
B genogroup	**D**	0		4 (6.7)	4 (6)
	Da	1		8 (13.4)	9 (13.4)
	E	3		20 (33.3)	23 (34.3)
	Subtotal	4		32 (53.4)	36 (53.7)
Intermediate genogroup	**F**	1		15 (25)	16 (23.9)
	G	0		2 (3.3)	2 (3)
	Subtotal	1		17 (28.3)	18 (26.9)
C genogroup	**H**	0		0	0
	I	2		4 (6.7)	6 (9)
	Ia	0		5 (8.3)	5 (7.5)
	J	0		2 (3.3)	2 (3)
	K	0		0	0
	Subtotal	2		11 (18.3)	13 (19.4)
Total		7		60 (100)	67 (100)

Table 1: Distribution of *C. trachomatis* genotypes in women and men, as determined by *omp1* nested PCR-based RFLP genotyping

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		B genogroup (n=36) n (%)	Intermediate genogroup (n=18) n	C genogroup (n=13) n	P
Sex	Male	4 (11.1)	1	2	0.6**
	Female	32 (88.9)	17	11	
Age	<=20	10 (27.8)	10	4	0.12**
	]20-25]	13 (36.1)	3	6	
	]25-30]	5 (13.9)	4	3	
	>30	8 (22.2)	1	0	
	Mean/Std.dev	25.1 ± 7.6	22.8 ± 6.3	22.1 ± 4.1	0.28***
	Min-Max	15-44	16-42	13-30	
Clinical symptoms	Absent	1 (2.9)	1	1	0.76**
	Present	33 (97.1)	17	12	
Pruritis	Absent	21 (63.6)	10	4	0.19*
	Present	12 (36.4)	7	8	
Pelvic pain	Absent	23 (69.7)	10	8	0.72**
	Present	10 (30.3)	7	4	
Discharge	Absent	6 (18.2)	2	2	0.9**
	Present	27 (81.8)	15	10	
Urethritis	Absent	30 (90.9)	16	10	0.72**
	Present	3 (9.1)	1	2	
Genital infections					
N. gonorrhoeae	Absent	35 (97.2)	18	9	0.005**
	Present	1 (2.8)	0	4	
Vaginosis	Absent	18 (50.0)	10	5	0.64*
	Present	18 (50.0)	8	8	
T. vaginalis	Absent	34 (94.4)	18	12	0.58**
	Present	2 (5.6)	0	1	
Candida sp.	Absent	34 (94.4)	17	12	1**
	Present	2 (5.6)	1	1	
HIV	Absent	30 (88.2)	18	13	0.23**
	Present	4 (11.8)	0	0	

425 *Chi-squared test, ** Fisher's exact test, *** Analysis of variance test

426 **Table 2: Clinical and laboratory data as a function of *C. trachomatis* genogroup**

	N (%) of					
	Men			Women		
Serological data	**CT+ (n=9)**	**CT- (n=21)**	**p**	**CT+ (n=65)**	**CT- (n=238)**	**p**
IgA-negative	4	17	0.05*	29 (44.6)	182 (76.5)	<0.0001*
IgA-positive	5	4		36 (55.4)	56 (23.5)	
Mean IgA ratio	2.1±1.6	0.8±0.43	0.002***	1.6±1.4	1.0±1.1	0.0002***
Min-Max	0.5-5.32	0.39-2.13		0.27-8.02	0.14-12.34	
IgG-negative	1	14	0.005*	13 (20.0)	131 (55.0)	<0.0001*
IgG-positive	8	7		52 (80.0)	107 (45.0)	
Mean IgG ratio	3.0±2.1	1.3±1.5	0.02***	4.5±3.1	2.0±2.2	<0.0001***
Min-Max	0.7-7.56	0.38-5.48		0.41-11.6	0.22-11	
IgA+/IgG+	5	3	0.02**	35 (53.8)	45 (18.9)	<0.0001**
IgA-/IgG+	3	4		17 (26.2)	62 (26.0)	
IgA-/IgG-	1	13		12 (18.5)	120 (50.4)	
IgA+/IgG-	0	1		1 (1.5)	11 (26.1)	

* Chi-squared test, ** Fisher's exact test, *** Student's t test

Table 3: Serological data in men and women as a function of positive and negative results for *C. trachomatis* detection in the Gen-Probe AMP CT assay

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Serological data	B genogroup (n=26) n	Intermediate genogroup (n=10) n	C genogroup (n=10) n	P
IgA-negative	10	3	7	0.15*
IgA-positive	16	7	3	
Mean IgA ratio	1.44±0.93	1.6±0.74	1.87±2.47	0.70**
Min-Max	0.27-3.45	0.72-3.07	0.34-8.02	
IgG-negative	2	0	6	0.001*
IgG-positive	24	10	4	
Mean IgG ratio	5.5±2.9	6.5±3.8	2.3±2.6	0.008**
Min-Max	0.74-10.46	1.1-11.6	0.41-7.39	
IgA+/IgG+	16	7	3	0.007*
IgA-/IgG+	8	3	1	
IgA-/IgG-	2	0	6	

434 * Fisher's exact test, **Analysis of variance

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436 **Table 4: Serological data as a function of *C. trachomatis* genogroup**

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