Absence of Swedish New Variant *Chlamydia trachomatis* (nvCT) and *C. trachomatis* Genotype Distribution in Gipuzkoa, Spain, 2009–2010

Luis Piñeiro[1], Magnus Unemo[2] and Gustavo Cilla[1]

[1]Servicio de Microbiología, Hospital Donostia, Paseo Dr. Beguiristain s/n, ES-20014 San Sebastián, Gipuzkoa, Spain and [2]Swedish Reference Laboratory for Pathogenic Neisseria, Department of Laboratory Medicine, Clinical Microbiology, Örebro University Hospital, Örebro, Sweden. E-mail: luisdario.pineirovazquez@osakidetza.net

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The reported incidence of sexually transmitted *Chlamydia trachomatis* (CT) infections has recently increased substantially in many European countries, probably due to the introduction of more sensitive diagnostic methods, e.g. nucleic acid amplification tests (NAATs), and enhanced CT transmission, as well as improved screening and reporting systems (1). In 2006 a new variant of *C. trachomatis* (nvCT) was reported in Sweden. The nvCT contains a 377-base pair (bp) deletion in the cryptic plasmid that covers the single targets originally utilized in the NAATs from Roche Diagnostics (Amplicor, Cobas Amplicor, Cobas TaqMan48) and Abbott Laboratories (Abbott *m*2000) (2, 3). The nvCT caused many thousands of falsely negative diagnoses in Sweden (4–6). Although the nvCT has been detected in Norway, Finland and Denmark (5–7), only a few cases of nvCT have been reported outside the Nordic countries (5, 6, 8, 9). Knowledge of nvCT presence and spread in many countries, especially those in southern Europe, remains limited, since only a few studies have been reported (5, 10, 11). No screening for nvCT has been performed previously in Spain, despite the fact that more than one million people from Sweden (and numerous others from the other Nordic countries) travel to Spain each year.

The aims of this study were to analyse the possible presence of nvCT and to describe the genotype distribution of CT in Gipuzkoa (Basque Country, northern Spain).

MATERIALS AND METHODS

All samples included (Table I) were analysed at the University Hospital of Donostia, San Sebastián, Gipuzkoa, which has a catchment population of 405,745 inhabitants. The samples (mainly urethral, cervical and rectal swabs) were from symptomatic patients with suspected sexually transmitted infections (STI) attending our hospital or one main STI clinic, or from asymptomatic patients (approximately one-third) with risk exposures consulting for a routine check-up. Between January 2006 and November 2008, CT detection was performed using the COBAS TaqMan CT Test (Roche Diagnostics, Branchburg, NJ, USA), which detects wild type CT (wtCT) but not nvCT. From December 2008 to December 2010, the new COBAS TaqMan CT Test v2.0 (Roche Diagnostics) was used. This dual target test, which targets both the cryptic plasmid and the chromosomal *ompA* gene, detects both wCT and nvCT (as well as plasmid-free CT mutants). Detection and *ompA* genotyping of positive samples were performed as described previously (12). For detection of nvCT, DNA isolation with the robotized system NucliSens easyMAG (bioMérieux, Boxtel, The Netherlands) and a previously described real-time PCR (3), slightly modified by using melting curve analysis instead of hybridization probes, were used. This PCR easily distinguished between wCT (melting temperature approximately 81ºC) and nvCT (approximately 78ºC). For confirmation, all amplicons were sequenced. Purified DNA from the previously genome-sequenced nvCT (Sweden2 (6)) was included as a positive control in all PCRs.

Table I. *Total number, percentage of positive samples and proportion of genotype E* Chlamydia trachomatis*, by year and in the 2 study periods*

Year/month	Samples *n*	Positive *n* (%)	Typed *n* (%)	Genotype E *n* (%)
2006	405	31 (7.7)	24 (77)	10 (42)
2007	573	40 (6.9)	33 (83)	17 (52)
2008	820	70 (8.5)	59 (84)	31 (53)
2009	946	79 (8.4)	66 (84)	22 (33)
2010	1,039	82 (7.9)	73 (89)	30 (41)
Jan 2006–Nov 2008	1,721	135 (7.8)	112 (83)	56 (50)
Dec 2008–Dec 2010	2,062	167 (8.1)	143 (86)	54 (38)
Total	3,783	302/3783 (8.0)	255/302 (84)	110/255 (43)

RESULTS

During the study period, the number of samples submitted for CT diagnostics increased substantially, although the percentage of positive samples remained largely constant (Table I). From December 2008 to December 2010, the period in which molecular assays allowed the presence of both wtCT and nvCT to be analysed, 167 (8.1%) of the 2,062 samples analysed were positive. Of these positive samples ($n = 167$), 143 (86%) could be genotyped and 54 (38%) of these belonged to genotype E (51 episodes from 50 patients (24 men and 26 women), median age 30 years (range 20–60 years)). No nvCT or strains lacking the cryptic plasmid were found. In the amplified sequence, the 54 wCT strains showed a nucleotide sequence identity of $\geq 99\%$ among each other and the prototype strain Sweden3 (GenBank accession number FM865440). Throughout the study period, the most frequent genotype was genotype E, followed by F, D and G, with no significant differences between the two periods in genotypes E, D and G (Table II). In contrast, the proportion of genotype F samples increased from 9.4% ($n = 10$) in the first period to 18.5% ($n = 25$) in the second (χ^2, $p = 0.046$). Interestingly, the L2b genotype was detected in rectal swabs from two homosexual men with proctitis, and genotypes B and A, which have rarely been described in genital infections (13), in urethral samples from three men with urethritis (GenBank accession number of one strain of each genotype: L2b-JF812080, B-JF812071, A-JF812081).

Table II. *Number and percentage of episodes of* Chlamydia trachomatis *infection genotyped in the two study periods (samples of a patient obtained in ≤6 months were considered as the same episode)**

	Genotype, *n* (%)											
Study period	E	G	D	F	I	J	K	H	L	B	A	Total
Jan 2006 – Nov 2008	52 (49.1)	14 (13.2)	12 (11.2)	10 (9.4)	7 (6.6)	6 (5.7)	2 (1.9)	1 (0.9)	1 (0.9)	1 (0.9)	0	106
Dec 2008 – Dec 2010	51 (37.8)	17 (12.6)	23 (17.0)	25 (18.5)	4 (3.0)	6 (4.4)	2 (1.5)	4 (3.0)	1 (0.7)	1 (0.7)	1 (0.7)	135

*Additionally 14 samples were analysed, whose genotypes were the same as the genotype found in the other samples of their respective episodes.

DISCUSSION

In a globalized world with intense population movement (migrations, commerce, tourism, etc.), monitoring the molecular evolution and epidemiology of pathogenic microorganisms is essential to detect the emergence and spread of variant strains that change their behaviour (fitness, virulence, resistance phenotypes, detectability with distinct diagnostic methods, etc.) and to adopt effective preventive, diagnostic and therapeutic measures. The present study was based on the hypothesis that the substantial increase in the number of samples submitted for CT diagnostics and the number of CT-positive samples detected, when introducing dual target CT test, in our region (Table I) could be due partly to the recent emergence of nvCT. Moreover, more than one million people from Sweden (and many people from other Nordic countries) travel to Spain each year, which, like several other southern European countries, is a major destination for tourism and business. Furthermore, nearly 200,000 Spanish people (an estimated 4,000 from Gipuzkoa) travel to Sweden and the other Nordic countries each year. Nevertheless, no information has been reported previously on the presence or absence of nvCT in Spain, nor, with the exception of France, in any of the southern European countries (14). No nvCT was detected in Gipuzkoa (Basque Country, northern Spain), which receives approximately 8,000 tourists each year from the Nordic countries. However, it would also be valuable to perform the same type of nvCT screening at the main travel destinations in Spain (the Canary Islands and Mediterranean regions) and other countries, i.e. where most Swedish people go for holidays, a context in which sexual contacts may increase.

In conclusion, no nvCT was found in Gipuzkoa, Spain. However, nvCT causes disease and has the same biological fitness as wCT, and thus it may be expected to spread to other countries (5, 6). Epidemiological and microbiological surveillance, including nvCT screening, must be maintained through the use of appropriate diagnostic methods and external and internal quality assessments.

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The authors declare no conflict of interest.

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