Results of a Europe-wide investigation to assess the presence of a new variant of Chlamydia trachomatis

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In 2006, a new variant of *Chlamydia trachomatis* was reported in Sweden. Three countries – Ireland, Norway, and Denmark – have detected the variant to date, but very few cases in total have occurred. The European network for STI surveillance (ESSTI) and the European Centre for Disease Prevention and Control (ECDC) assessed the potential spread of the variant in other European countries, and concluded that there is currently no evidence that the variant has spread widely across Europe. However, the variant strain has been reported in between 10% and 65% of infected patients in Sweden. It is too early to tell whether the variant will remain confined to Sweden or whether the number of cases will significantly increase. Enhanced surveillance will need to be continued to address these concerns.

Introduction

In 2006, the occurrence of a new variant of *Chlamydia trachomatis* was reported in Sweden [1,2]. The variant had been detected following an unexpected 25% decrease in the number of infections observed in Halland county, southwest Sweden. The variant contains a 377 base pair deletion in the cryptic plasmid, which is the region targeted by the nucleic acid amplification tests (NAAT) manufactured by both Cobas Amplicor, Cobas Taqman48 and Abbott m2000 (manufactured by Roche and Abbott) [1] Patients infected with this variant of *C. trachomatis* would therefore be given a false negative result if a laboratory used either of these assays as its diagnostic test. Other commercially available NAAT tests such as the the ProbeTec Strand Displacement Assay (SDA) (Becton Dickinson), Aptima Combo 2 (AC2) test (Genprobe), and RealArt CT Kit (Qiagen), target other areas of the cryptic plasmid, the 16SrRNA and omp gene respectively and therefore will detect this variant of *C. trachomatis*.

Genital chlamydial infection is the most prevalent bacterial sexually transmitted infection (STI) in many European countries [2] and any reduction in the detectability of infection has potential implications for public health in Europe. Initial data from Sweden showed that 39% of all chlamydia cases detected during one month were caused by the new variant of *Chlamydia trachomatis* [3]. Although the data from Sweden is so far limited, if this turns out to be the true representative proportion of chlamydial infection by the genetic variant in Sweden and this is then replicated across Europe, an inability to detect such a sizeable proportion of chlamydia infection could have serious consequences. Therefore, the European network for STI surveillance (ESSTI) and the European Centre for Disease Prevention and Control (ECDC) decided to assess the potential spread of the new variant in other countries across Europe [4].

Methods

ESSTI and ECDC designed a short survey to address this issue (A copy of the questionnaire is available upon request from the corresponding author). The questionnaire contained six questions and was sent with a cover letter to all ESSTI collaborators, both epidemiologists and microbiologists, in 25 countries (22 EU member states and Iceland, Norway and Turkey) in February 2007. The survey collected information on the type of NAATs used to diagnose chlamydia, the extent to which NAATs are used for chlamydia diagnosis and also requested information on any actions or investigations that a country or laboratory undertook in response to the appearance of the new variant. Finally, a question asked whether guidelines regarding the diagnosis of chlamydia in the respective countries had been issued or changedissued.

Results

In total, 21 countries had responded to the request by the beginning of May 2007, but only 19 were able to provide any information. Four countries did not respond. Several countries submitted more than one questionnaire, as the survey provided the option of describing information either for the whole country, a particular region or for an individual laboratory; two questionnaires were returned from Portugal and Estonia andthree from Slovenia and England, while Ireland provided results from 17 individual laboratories. Ten countries provided information for the whole country, seven provided information from individual laboratories, Finland gave information from a particular region and Estonia submitted data both from a regional source and an individual

laboratory. Seventy-five percent of respondents (n=24/32) based their answers on actual laboratory data.

Table 1 describes the level of NAAT testing for chlamydia in the countries belonging to the ESSTI network and the number of chlamydia diagnoses. The proportion of chlamydia diagnoses performed by NAAT where information was available for the whole country ranged from 12% in Cyprus to 100% in Iceland, Malta, Netherlands, Norway and Scotland. Malta and Iceland were the only countries that used the Cobas Amplicor or Cobas Taqman48 exclusively, although there was widespread use of this test in individual laboratories in other countries (Figure 1). The Abbott m2000 assay which is also unable to detect the variant was only used in three countries and accounted for only a small number of routine diagnostic tests; France (<5%), Netherlands (5%) and Sweden (3.8%).

Twelve respondents reported that action was or is in the process of being undertaken in their country to assess whether the variant was present. Countries used one or a combination of the following three approaches: Retrospective testing, dual testing and monitoring of surveillance data (Table 2). Retrospective testing of samples was carried out in Denmark, France, Sweden, England, Finland and the Netherlands with varying approaches either by retesting specimens that had tested negative using a test unable to detect the variant or retesting samples that originally tested positive by a test known to detect the variant. New national guidelines for testing were issued in Sweden for laboratories changing from the Cobas Amplicor, Cobas Taqman48 and Abbott m2000 to other tests such as the ProbeTec Strand Displacement Assay (SDA) (Becton Dickinson). In Denmark, the National Board of Health wrote to laboratories recommending that they should either change to a method which could detect the mutant or to forward the specimen to another laboratory.

Table 1

Information provided by country, region and laboratories on proportion of chlamydia testing by NAAT and total number of tests and diagnoses in 2006

Country	Proportion of chlamydia cases diagnosed by NAAT (%)	Proportion based on data or estimate	No. of NAAT tests	No. of chlamydia diagnoses by NAAT	Action taken to investigate presence of variant?
Country Level					
Cyprus	12	Estimate	530	61	No
Denmark	99.9	Data	324431	24866	Yes
France	63[1]	Estimate	500000	NK	Yes
Iceland	100	Data	17202[2]	1641[2]	Yes
Malta	100	Data	1106	46	No
#Netherlands	100	Estimate	57892	5989	Yes
#Norway	100	Data	273741	19973	Yes
#Scotland	100	Data	222709	17289	Yes
Sweden	95	Data	427551	30892	Yes
Turkey	70	Estimate	188	2	No
Region Level					
Estonia 1	85	Estimate	36209	1905	No
Finland	100	Data	53000	3169	Yes
Laboratory Level					
Austria	100	Data	20000	740	Yes
Belgium	100	Data	2075	138	No[3]
England 1	100	Data	22964	1936	Yes
England 2	100	Data	95500	9200	Yes
Estonia 2	100	-	13500	2500	No
[4]Ireland 1	100	Data	20000	2000	Yes
Ireland 2	100	Data	15895	NK	No
Ireland 3	100	Data	8180	666	No
Ireland 4	100	Data	1800	143	No
Ireland 5	100	Data	1361	103	No
Ireland 6	100	Data	2766	99	No
Ireland 7	100	Data	24005	1687	No
Ireland 8	-	-	60	0	-
Ireland 9	100	Data	1320	92	No
Portugal 1	100	Data	2768	222	Yes
Portugal 2	100	Estimate	4200	263	No
Slovak Republic	100	Data	5306	10	No
Slovenia 1	70	Data	1007	122	No
Slovenia 2	48.9	Data	263	27	No
Slovenia 3	12.6	Data	22	NK	No

Data from 2005

[1]Private Laboratories only

[2]Data from 1 lab only. One other lab does chlamydia testing but total number of tests is unknown.

[3]Laboratory routinely screens with SDA ProbeTec and Amplicor. No increase in discordant results seen.

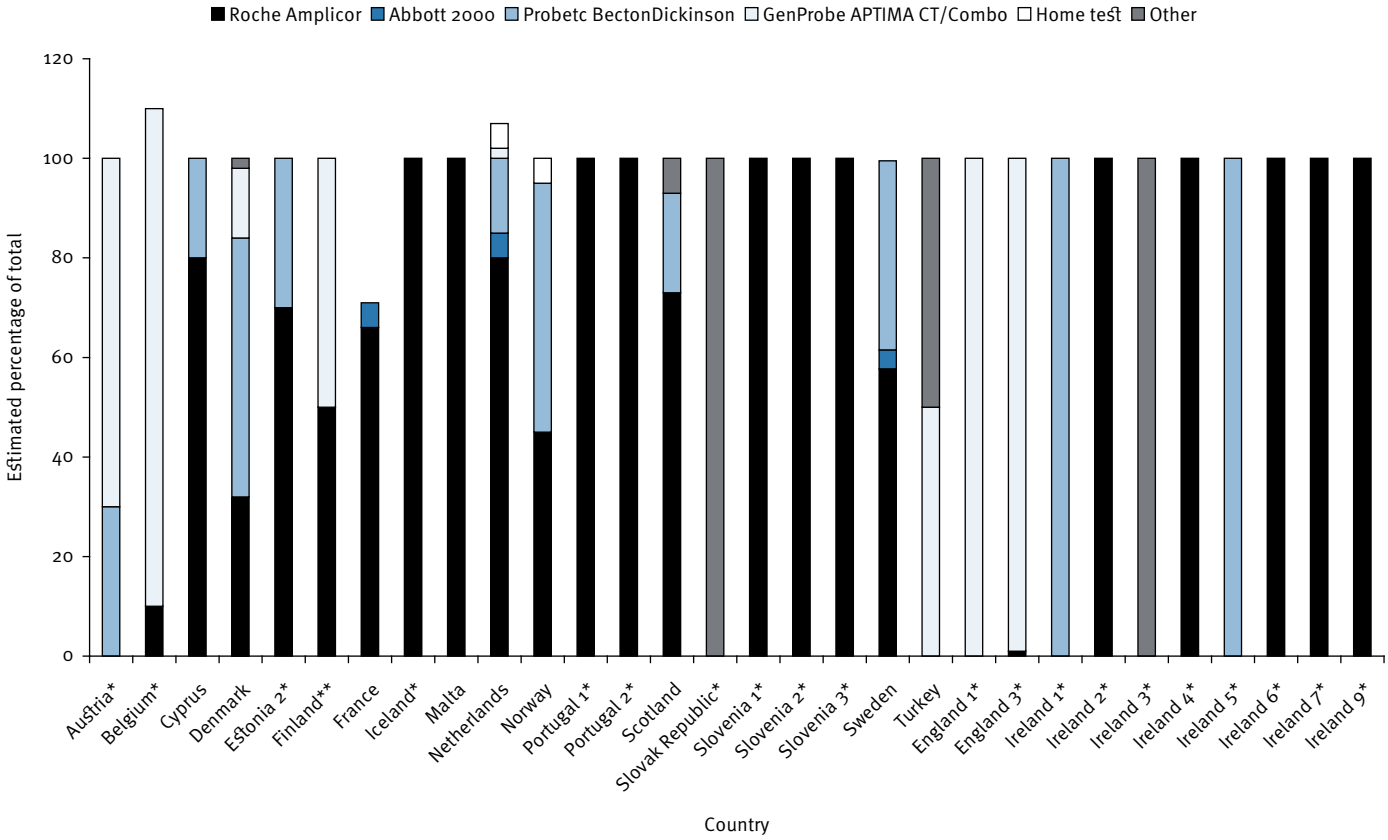
[4] A further 8 labs surveyed in Ireland do not carry out chlamydia testing

- Missing data

NK: Not Known

Figure 1

NAATs used for routine diagnosis of C. trachomatis



Discussion

This survey attempted to assess the potential spread of the new variant across Europe. However, it was not feasible in the available timeframe to survey directly all laboratories, both private and public, that carry out chlamydia diagnostics across Europe. A possible bias in the survey is that data is more likely to have been obtained from public laboratories, but there is no reason why the type of tests used would differ significantly in private laboratories. Although the results of this survey can not be considered comprehensive for all European countries, 10 respondents were able to provide information for the whole country and a further four countries surveyed more than one laboratory. The coverage obtained is therefore considered sufficient to determine whether the variant has spread outside Sweden to a great extent. Since the first report of the new variant, several European countries have undertaken extensive investigations to determine whether the variant is present in their country. Despite this active surveillance, only three countries – Ireland, Norway, and Denmark – have detected the variant to date and very few cases in total have occurred. Two cases of the new variant have been reported in both Norway and Ireland. One of the cases in Norway was of Swedish origin [5]. Similarly, in Ireland, one of the two cases, who were partners, was also of Swedish origin [6]. Since the questionnaire was completed, a single case of the variant has also been detected in Denmark – this case had no known link to Sweden [7]. Further epidemiological information on these cases of the new variant is currently unknown.

There is therefore no existing evidence that the variant has spread widely across Europe even into neighbouring countries and yet in Sweden the variant strain has been reported in between 10% and 65% of the total number of infected patients [8,9]. It is not known when the variant first appeared in Sweden but the increase in prevalence has been both rapid and recent. In Sweden, a considerable increase in chlamydial infection (53%) was reported in the first six months of 2007, compared to the same period in 2006. (Blaxhult, abstract isstdr page 391). It is unclear why the variant is present in such a sizeable proportion of cases in Sweden and yet has not made an impact in other countries. It may be present at very low levels in other countries but the results of the survey suggest that, following the extensive search for the variant by many countries, it would have been detected if it was present in the testing population. A possible reason why the variant does not appear to have spread outside Sweden may be found in a study carried out in one county in Sweden which reported that 79% of all sexual partners of chlamydia cases lived within 100km of each other [8]. Sex abroad may not be a significant risk factor for the acquisition and hence spread of chlamydia infection unlike in the case of other STIs such as syphilis where it is well documented. In Sweden, it has been hypothesised that a number of factors are present that may have resulted in selection of the variant, for example the high number of diagnostic tests carried out almost exclusively by the Roche assay, the lack of contact tracing performed for false negative persons and the treatment of symptomatic patients only [10].

TABLE 2

Details of Investigations carried out across Europe

Country	Type of Investigation	Initial Test Used	Current Test Used	Population or setting	Sample size
Austria	Dual Testing	NK	NK	NK	N=300-400
Denmark	Retrospective Testing of specimens found positive or negative at other laboratories	Cobas Amplicor/, Taqman48 (Roche), (ProbeTec (SDA, Becton Dickinson)	Three SSI Chlamydia trachomatis-PCR methods	5 Departments of Clinical Microbiology	N=1077
	Dual Testing	NK	Three SSI Ct-PCR methods	Since October 2006 3 methods have been used for all Ct-PCRs at SSI, 1 of which can identify the mutant	N=2620
	Data: examine no. positive by month by method from 1 Jan 2004 to 31 Dec 2006	NK	NK	National mandatory surveillance registry of laboratory diagnosed chlamydia	2004: 21624 2005:23854 2006:24866
England	Retrospective Testing of previously negative samples	Cobas Amplicor/, Taqman48 (Roche)	Aptima Combo 2 (AC2) test (Gen-Probe)	1) Newcastle area 2) Genitourinary Medicine clinic	1)n=683 2) n=>1000
	Retrospective Testing of previously positive samples	Unaffected platform	In-house nested block based PCR assay	1) MSM patients from 30 GUM clinics 2) Primarily heterosexual patients from London, Portsmouth, Plymouth, Harrogate, Nottingham	1) n=179 2) n=933
Finland	Retrospective Testing	NK	NK	NK	NK
France	Retrospective Testing of previously negative samples	Cobas Taqman48, (Roche)	Cobas Taqman48, (Roche)and omp2 home test	Male and Female with risk factors	N=40
	Retrospective Testing of previously positive samples	An unaffected platform	Cobas Taqman48, (Roche)	Samples from a private lab which receives nationwide samples	N=500 in 2006
	Dual Testing	NK	Cobas Taqman48, (Roche)and omp2 home test	1)STD/HIV testing clinics in Bordeaux 2) Family planning clinics in Paris 3)Private lab in Paris dealing with mainly MSM 4)Adolescent clinic	1) n=252 male, 199 female 2) n=100 3) n=100 4) n=132
	Prospective testing during 2007	An unaffected platform	Cobas Taqman48, (Roche)	Samples from a private lab which receives nationwide samples	In progress
Iceland	Dual Testing	Cobas Amplicor/, Taqman48 (Roche)	Cobas Amplicor/, Taqman48 (Roche) and Becton Dickinson	All urine samples sent to Dept of microbiology Feb-May 2007	Approx n=4000
Ireland	Dual Testing	ProbeTec (SDA, Becton Dickinson) and Cobas Amplicor/, Taqman48 (Roche)	ProbeTec (SDA, Becton Dickinson) and Cobas Amplicor (Roche)	Department of Microbiology, St James Hospital, Dublin	NK
Netherlands	Retrospective Testing of previously positive samples	Validated in-house Taqman assay	NK	Academic centre	NK
	Dual Testing	NK	Cobas Amplicor/, Taqman48 (Roche)) and ProbeTec (SDA, Becton Dickinson)	High-risk attendees from 3 STI clinics in Amsterdam	NK
Norway	Data: no. tested/no. positive by test method	NK	NK	NK	NK
Portugal	Dual Testing	Cobas Amplicor/, Taqman48 (Roche)	Cobas Amplicor/, Taqman48 (Roche)	1 large private lab	N=4200
	Data: examine no. tested/no. positive by month in 2005 and 2006 Dual Testing when justified by clinical signs	Cobas Amplicor/, Taqman48 (Roche)	Cobas Amplicor/, Taqman48 (Roche) and ompA home test	Chlamydia-Neisseria Laboratory of the Portugese National Institute of Health	N=2768
Scotland	Dual Testing	NK	Cobas Amplicor/, Taqman48 (Roche)and Aptima Combo 2 (AC2) test (Gen-Probe)	1 large testing lab	N=3000
Sweden	Retrospective Testing of previously negative samples	Cobas Amplicor/, Taqman48 (Roche)Taqman48 and Abbott m2000	ProbeTec (SDA, Becton Dickinson)	Some laboratories	NK
	Dual Testing	NK	Cobas Amplicor/, Taqman48 (Roche) and Abbott m2000 and ProbeTec (SDA, Becton Dickinson)	Some laboratories	NK

NK: not known

Conclusions

The presence of a *C. trachomatis* variant that is not detectable, hence causing false negative test results, has serious implications for patient management, care and the transmission of *C. trachomatis* in the population. Therefore, experts in all EU Member States should remain vigilant. More epidemiological information regarding the affected population needs to be collected in order for targeted public health measures to be undertaken. The emergence of the variant suggests it may be more appropriate for any NAAT to include dual targets which is being considered by test manufacturers [8]. It is too early to tell whether the variant will remain confined to Sweden or whether the number of cases will significantly increase. Enhanced surveillance will need to be continued to address these concerns. ESSTI and ECDC aim to repeat the survey at the end of the year to determine if the picture across Europe remains the same.

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