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Screening for *Chlamydia trachomatis* in secondary schools, family planning and occupational health centres in Luxembourg

Joël Mossong¹, Margot Muller², Nicole Majéry³, Claudine Mardaga⁴, Frédéric Decruyenaere¹, François Schneider¹

¹ Microbiology, National Health Laboratory, Luxembourg, Luxembourg

² Division of School Health, Health Directorate, Luxembourg, Luxembourg

³ Multisectorial Health at Work Services, Luxembourg, Luxembourg

⁴ Family Planning, Luxembourg, Luxembourg

* Author for correspondence: Dr J. Mossong, Laboratoire National de Santé, PO Box 1102, L-1011 Luxembourg, phone : +352 49 11 91 336, fax +352 40 42 38, email : joel.mossong@lns.etat.lu,

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Contributions of each author: JM designed the study, conducted the data analysis and wrote the manuscript; MM organised screening at the secondary schools and reviewed the manuscript; NM organised the screening at the Occupational Health Centre and reviewed the manuscript; CM organised the screening at the Family Planning Centres and reviewed the manuscript; FD and FS designed the inhouse test, conducted the laboratory testing and reviewed the manuscript.

Key messages

- Our study aimed to gauge the feasibility of screening for urogenital *Chlamydia trachomatis* infection in different health settings where routine urinary testing is conducted independently for other purposes
- Prevalence of urogenital chlamydial infection differed significantly between secondary schools, family planning and occupational health centres
- Depending on the setting, identified risk factors included being 18-22 years old, female sex, having three or more sexual partners in the past year, or inconsistent condom use
- As highest prevalence of infection was observed at the family planning centres and as provision of treatment, follow-up and repeat testing can be easily implemented therein, this setting seems best suited for conducting Chlamydia screening activities.

SUMMARY

Background. We conducted a prospective pilot screening study in three settings in Luxembourg where routine urine testing is carried out independently for other purposes with a short sexual behaviour questionnaire to estimate prevalence and determine risk factors of urogenital *Chlamydia trachomatis* (CT) infection.

Methods. Screening was offered to sexually active volunteer participants aged less than 25 years in three settings: i) women in 3 family planning centres (FPC), ii) young women and men in 29 secondary schools and iii) women and men in an occupational health centre (OHC) for newly recruited employees and workers. First catch urine samples were tested using the COBAS Amplicor and an in-house assay. Multiple logistic regression was performed to analyse risk factors.

Results. Overall prevalence among 4141 participants was 7.7% (95% confidence interval (CI) 6.3-9.2) in FPCs, 1.9% (95% CI 1.2-2.8) in secondary schools and 4.5% (95% CI 3.5-5.6) in the OHC. Depending on the setting, identified risk factors included being 18-22 years old, female sex, having three or more sexual partners in the past year, and inconsistent condom use

Conclusion. Screening is feasible in the three settings, but the prevalence of CT infection among men and women is highest in age groups that have left secondary school. Family planning centres were the setting with the highest CT prevalence and the only setting in our study able to provide case management, follow-up and repeat testing.

INTRODUCTION

Uro-genital *Chlamydia trachomatis* infection is the most commonly reported bacterial sexually transmitted infection in developed countries [1]. The majority of *C. trachomatis* infections are asymptomatic or lead to mild clinical symptoms such that no treatment is sought. Screening of asymptomatic men and women has been advocated because of the potential complications from untreated chlamydial infection in women including pelvic inflammatory disease (PID) leading to infertility or ectopic pregnancy [2], pre-term delivery and pre-mature rupture of membranes [3].

In the past decade, screening for chlamydial infection in non-clinical settings has been facilitated by the availability of sensitive commercial nucleic-acid amplification techniques applied to non-invasive urine samples [4]. As a result of these, a number of countries have formulated screening guidelines or implemented control measures [5]. The U.S. Preventive Services Task Force in the United States, currently recommends screening for chlamydial infection for all sexually active women aged 24 years or younger as well as older pregnant women at increased risk [6]. Whether and how screening should be organised remains an issue of considerable debate [7].

In Luxembourg, to our knowledge, there have been no previous studies published on urogenital *C. trachomatis* prevalence and there are currently no official screening or treatment guidelines available to clinicians.

The principal aim of our study was to gauge the feasibility of chlamydial screening in three different settings (family planning centres (FPCs), secondary schools and occupational health centre (OHC)) where routine urine testing is carried out independently for other purposes, e.g. pregnancy test at FPCs, screening for diabetes and urinary tract infection at the other two settings. We estimated the prevalence of urogenital *C. trachomatis* infection in these populations and identified risk factors for chlamydial infection to be able to provide policy makers with evidence-based recommendations for screening activities.

METHODS

Study design

The study was conducted in three settings in Luxembourg: i) women attending any of three family planning centres (FPCs) between August 2004 and November 2006; ii) male and female students aged 16 years or older attending a compulsory routine medical examination in 29 secondary schools in Luxembourg between October 2004 and June 2005; and iii) women and men less than 25 years old attending an occupational health centre (OHC) from November 2005 to November 2006. An occupational health check at the start of the working contract and periodic health visits for certain risky jobs are mandatory for all employees and workers in Luxembourg.

An information leaflet describing *C. trachomatis* infection and the study purpose was handed to study participants and the study was explained to them. After giving informed written consent, participants were asked to fill out a questionnaire with basic demographic details, sexual behaviour and sexual history and to provide a first void urine sample in 50 ml polypropylene containers. For study participants recruited at FPCs, test results were communicated either by phone or mailed to an address as indicated by the participant. Positive participants were advised to return to one of the FPCs for free treatment including free expedited partner therapy.

In secondary schools, test results were handed out individually by the school health teams and positive students were advised to either seek free treatment at one of the FPCs or to visit their general practitioner. Students with negative tests results or who were not sexually active were given a leaflet on the prevention of sexually transmitted diseases and condoms were offered free of charge.

For participants at the OHC, results were communicated by mail to the address indicated by the participant. Positive OHC participants were advised to seek treatment either free of charge at one of the FPCs or to see their GP for a prescription. Treatment for positive participants

and their partner(s) at FPCs consisting of 1 g of oral azithromycin was provided free of charge.

Laboratory methods

Urine samples were transported immediately to the laboratory and refrigerated at 4 degrees Celsius. Testing was accomplished within 24 hours using the COBAS AMPLICOR CT assay (Roche Diagnostic Systems, Branchburg N.J., USA) according to the manufacturer's instruction. All samples were also tested in parallel by an in-house real-time PCR assay on the LightCycler platform (Roche Applied Science) using primers described previously [8] with the modification that the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) was used for DNA extraction. Overall agreement between the two methods was 99.0% and the fraction of indeterminate results was low by either method (0.39% for COBAS AMPLICOR and 0.05% for the real-time method). For the purpose of this study we considered as positive those samples which were positive by either one of the two methods. Sixteen samples indeterminate by one method and negative by the other were considered as negative.

Statistical Analysis

95% confidence intervals (CI) for proportions were calculated using the exact binomial method. Univariate and multiple logistic regression were performed with positive chlamydial test as the dependent variable and sex, age, setting, reported number of partners in the past 12 months and reported condom use as independent variables. The goodness of fit of the model was assessed using the Hosmer-Lemeshow test. All statistical calculations were performed using Intercooled Stata 10.0 (College Station, TX, USA).

Ethical approval

Ethical approval for the study was obtained by the national research ethics committee.

RESULTS

Participation rates

Between October 2004 and June 2005, 3413 students in 29 secondary schools were eligible for screening. 222 students (6.5%) were absent on study day and 1172 (34.3%) declined participation. Overall, 2019 (59.2%) students consented to take part by filling in a questionnaire and/or giving a urine sample. 684 students (33.9% of participants) indicated not being sexually active and their urine sample was not tested. Using intention to screen analysis, 1327 were tested yielding an overall participation rate of 38.9%. At the occupational Health Centre, of 2480 clients invited to take part in screening 1458 agreed yielding a participation rate of 58.8%. No formal participation data was available for FPCs - an informal estimation by FPC receptionists indicated high participation rates in excess of 75% in clients approached for screening.

Prevalence and risk factors by setting

The overall prevalence of CT infection among 4141 participants was 4.4% but differed significantly between the three study sites ($p < 0.001$), ranging from 7.7% (95% CI 6.3-9.2) in FPCs, 4.5% (95% CI 3.5-5.6) in the OHC to 1.9% (95% CI 1.2-2.8) in secondary schools. Table 1 shows prevalence estimates separately for all study 3 sites as a function of sex, age, number of partners in the past 12 months and condom use. Table 2 shows results from univariate and multivariate logistic regression analysis separately for each site. In secondary schools, female sex (OR 3.8, 95% CI 1.3-10.8), age between 20-22 years (OR 7.1, 95% CI 1.8-28.0) and 3 or more sexual partners in the 12 previous months (OR 7.6, 95% CI 2.9-20.0) were independently associated with CT infection in multivariate analysis, but not condom use ($p = 0.109$). In FPCs, age between 18-19 (OR 2.0, 95% CI 1.1-4.0), age between 20 and 22 years (OR 1.8, 95% CI 1.0-3.2) and never using condoms (OR 2.6, 95% CI 1.2-5.6) were independently associated with higher CT prevalence in multivariate analysis, but not condom use. At the OHC, female sex (OR 2.1, 95% CI 1.2-3.7), 3 or more sexual partners in the 12

previous months (OR 2.6, 95% CI 1.3-4.9) and inconsistent (that is either often, sometimes or never) condom use (OR 4.1, 95% CI 1.3-13.2) were independently associated with higher CT prevalence in multivariate , but not age.

Table 1: Prevalence of *C trachomatis* infection by characteristic for the three study sites.

	Secondary schools		Family Planning		Occupational Health	
	positive	%	positive	%	positive	%
	/total	(95% CI [#])	/total	(95% CI [#])	/total	(95% CI [#])
Sex						
Women	20/794	2.5 (1.5-3.9)	104/1355	7.7 (6.3-9.2)	43/734	5.8 (4.2-7.8)
Men	5/534	0.9 (0.3-2.2)	-	-	22/724	3.0 (1.9-4.6)
Age group						
15-17	4/460	0.8 (0.2-2.2)	17/340	5.0 (2.9-7.9)	0/45	0 (0-7.9) ^{&}
18-19	16/769	2.1 (1.2-3.3)	33/303	10.9 (7.6-15.0)	7/174	4.0 (1.6-8.1)
20-22	5/98	5.1 (1.7-11.5)	41/428	9.6 (7.0-12.8)	32/596	5.3 (3.7-7.5)
23-25	0/1	0 (0-0.975 ^{&})	13/284	4.5 (2.4-7.6)	26/643	3.9 (2.6-5.8)
Partners in previous 12 months						
0 or 1	9/891	1.0 (0.5-1.9)	58/857	6.8 (5.2-8.7)	34/944	3.6 (2.5-5.0)
2	5/250	2.0 (0.7-4.6)	26/285	9.1 (6.0-13.1)	11/246	4.5 (2.2-7.9)
3 or more	11/184	6.0 (3.0-10.4)	14/168	8.3 (4.6-13.6)	18/237	7.6 (4.6-11.7)
NS*	0/3	0 (0-70.8) ^{&}	6/45	13.3 (5.0-26.8)	2/31	6.4 (0.8-21.4)
Condom use						
Always	3/480	0.6 (0.1-1.8)	9/206	4.4 (2.0-8.1)	3/249	1.2 (0.2-3.5)
Often	7/269	2.6 (1.1-5.3)	16/237	6.7 (4.0-10.7)	12/234	5.1 (2.7-8.8)
Sometimes	12/415	2.9 (1.5-5.0)	45/579	7.8 (5.7-10.3)	27/467	5.8 (3.8-8.3)
Never	3/161	1.9 (0.4-5.3)	29/285	10.2 (6.9-14.3)	21/477	4.4 (2.7-5.7)
NS*	0/3	0 (0-70.8) ^{&}	5/48	10.4 (3.5-22.7)	2/31	6.5 (0.8-21.4)
Total	25/1328	1.9 (1.2-2.8)	104/1355	7.7 (6.3-9.2)	65/1458	4.5 (3.5-5.6)

[#] CI - Confidence interval, binomial exact

[&] one-sided, 97.5% confidence interval

* NS- not stated, missing value

Table 2: Univariate and multivariate logistic regression analysis, conducted separately for each study site. Participants with missing values for number of partners or condom use (N=86) were excluded from analysis. P-values are based on the Wald test of respective coefficients in the regression model being simultaneously equal to 0.

	Secondary schools		Family Planning		Occupational Health	
	Univariate	Multivariate	Univariate	Multivariate	Univariate	Multivariate
	OR	OR	OR	OR	OR	OR ¹
Sex	p=0.046	p=0.013			p=0.011	p=0.007
Women	2.7 (1.0-7.3)	3.8 (1.3-10.8)	-	-	2.0 (1.2-3.4)	2.1 (1.2-3.7)
Men	1.0	1.0	-	-	1.0	1.0
Age group	p=0.027	p=0.019	p=0.015	p=0.006	p=0.513	p=0.44
15-17	1.0	1.0	1.0	1.0	-*	-*
18-19	2.4 (0.8-7.3)	2.3 (0.8-7.1)	2.1 (1.1-4.0)	2.0 (1.1-3.7)	1.0	1.0
20-22	6.2 (1.6-23.5)	7.1 (1.8-28.0)	1.9 (1.0-3.4)	1.8 (1.0-3.2)	1.3 (0.6-3.0)	1.2 (0.5-2.9)
23-25	-*	-*	0.9 (.4-1.9)	0.8 (0.4-1.7)	0.9 (0.4-2.3)	0.9 (0.4-2.0)
Partners in previous 12 months	p<0.001	p<0.001	p=0.401	p=0.109	p=0.0325	p=0.018
0 or 1	1.0	1.0	1.0	1.0	1.0	1.0
2	2.0 (0.7-6.0)	2.0 (0.6-6.2)	1.4 (0.9-2.2)	1.5 (0.9-2.4)	1.3 (.6-2.5)	1.3 (0.6-2.7)
3 or more	6.2 (2.5-15.2)	7.6 (2.9-20.0)	1.3 (0.7-2.3)	1.4 (0.8-2.7)	2.2 (1.2-4.0)	2.6 (1.3-4.9)
Condom use	p=0.109	p=0.416	p=0.119	p=0.0818	p=0.0668	p=0.116
Always	1.0	1.0	1.0	1.0	1.0	1.0
Often	4.2 (1.1-16.6)	2.6 (0.6-10.2)	1.6 (0.7-3.7)	1.4 (0.6-3.3)	4.4 (1.2-15.9)	3.6 (1.0-13.3)
Sometimes	4.7 (1.3-16.9)	3.0 (0.8-11.0)	1.8 (0.9-3.8)	1.8 (0.9-3.8)	5.0 (1.5-16.8)	4.5 (1.3-15.0)
Never	3.0 (.6-15.1)	2.8 (0.5-14.7)	2.5 (1.1-5.4)	2.6 (1.2-5.6)	3.8 (1.1-12.8)	3.8 (1.1-13.0)

* No positive cases for this category, odds ratio cannot be calculated

¹ Multivariate model for participants at occupational centre included only participant older than 18 years, as no participants in this setting below age of 18 were positive

DISCUSSION

To our knowledge, this is the first prevalence study on urogenital *C. trachomatis* infection in Luxembourg. From a practical point of view screening appears to be feasible in the three studied settings, but the prevalence of infection was highest in female participants aged 18-22 and this group should probably be the main target of any future screening activities. For this reason, and because treatment, follow-up and repeat testing of positive patients (and their partners) can be offered concurrently, FPCs appear to be the most suited of all three settings considered for screening activities. Population coverage is likely to be limited if screening is only to be offered in this setting: on a yearly basis, approximately 2500 women aged between 16 and 25 use FPC services, representing approximately 10% of women in this age group in Luxembourg. One of the main challenges for the future is to study how other sexually active women and men in this age group at higher risk could be included.. One way to improve coverage in a pragmatic way could be to combine *Chlamydia* screening efforts with existing cervical cancer screening programmes [17 18] although this again raises difficult organisational (e.g. different target age), economic and laboratory issues.

Another limitation of our study is that it is based on volunteer participation and this could lead to selection bias. Whereas participation rates were very high in FPCs where participants seek contact themselves for other reasons, screening at secondary schools and the OHC was based on a compulsory examination and voluntary participation in these settings was much lower. One of the major advantages of offering screening in secondary schools is the low additional recruitment costs, which have a substantial impact in cost-effectiveness analyses [9]. Because such periodic medical examinations are compulsory in all secondary schools, it is a unique opportunity to target all school-going sexually active students on a national scale providing equitable access to screening for both men and women. In addition, *Chlamydia* screening activities might provide an interesting educational backdrop for raising awareness about sexual and reproductive health and promote safer sex practices in general. Indeed,

approximately three quarters of participants in our study overall reported inconsistent condom use (see table 1) and thus do not protect themselves (and their partners) adequately from acquiring sexually transmitted diseases. There are however also substantial limitations for offering screening in secondary schools: prevalence was lowest of all settings but similar to what has been observed in similar demographic settings in other countries [10 11], a sizeable fraction of pupils at secondary school were either not sexually active, did not want to participate in screening, or were not available at the screening session - particularly students in vocational training spent a significant amount of time off campus.

The main rationale behind our pilot study was to offer screening in settings where urine testing was conducted independently for other purposes. In Luxembourg, occupational health checks which are mandatory for newly recruited employees and workers are indeed such an opportunity. The fact that prevalence in this settings exceeded prevalence in secondary schools suggests that screening should also be considered, because it allows targeting young men in addition to young women. However, both the school and OHC settings suffer from the disadvantage that, for legal reasons, no medical treatment and follow-up can be offered on-site, and positive patients need to seek medical treatment elsewhere. It would have been interesting to estimate the proportion of positive participants actually receiving treatment in these settings, but our study design did not foresee this. Furthermore repeat testing in these sites cannot be easily implemented: in secondary schools, the mandatory medical examination are carried out every two years and at the OHC, periodic examinations are only carried out for workers with a high occupational risk.

One of the remaining issues, and one our study was not designed to address, is whether screening should be offered from a cost-effectiveness point of view. A review of published studies on cost-effectiveness in other countries suggested that threshold population prevalence of *C trachomatis* over which economic evaluations were cost-effective varied from 3.1-10.0% [12], which would mean that screening in Luxembourg should at best be only offered in

family planning and occupational health centres, but not in secondary schools. However, several authors have called for caution on using these thresholds for two reasons: first, results from previous cost-effectiveness studies depended critically on assumptions of high incidence of Chlamydia-associated pelvic inflammatory disease, which has been questioned recently [5 13-15] and second, the vast majority of cost-effectiveness analyses have relied on static instead of dynamic transmission models and this leads to unreliable conclusions [16].

Our study is the first to document asymptomatic urogenital *Chlamydia trachomatis* infection in young women and men in Luxembourg and confirms traditional risk factors (age, sex, high number of sexual partners and condom use) in different settings. While we identified screening to be technically feasible in all three settings, offering screening at each setting comes with its own advantages and disadvantages. Because provision of treatment, follow-up and repeat testing are integral aspects of any screening activity, family planning centres seem to be best suited for screening. The results of this study were presented to the Ministry of Health which did not issue any guidelines on screening, but agreed to fund continuing opportunistic screening at the family planning centres.

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