Impact factor 5.49

Eurosurveillance

Europe's journal on infectious disease epidemiology, prevention and control

Vol. 18 | Weekly issue 28 | 11 July 2013

ecdc EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

www.eurosurveillance.org

Control of recent community-based outbreaks of invasive meningococcal disease in men who have sex with men in Europe and the United States

D Weiss (Dweiss@health.nyc.gov)[1], J K Varma[1]
1. New York City Department of Health and Mental Hygiene, New York, United States

Citation style for this article:
Weiss D, Varma JK. Control of recent community-based outbreaks of invasive meningococcal disease in men who have sex with men in Europe and the United States. Euro Surveill. 2013;18(28):pii=20522. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20522

Article submitted on 10 July 2013 / published on 11 July 2013

Invasive meningococcal disease (IMD) is an infrequent yet deadly infection that constitutes a public health emergency. Control requires rapid identification and diagnosis of suspect cases, prompt administration of antibiotic prophylaxis to close contacts, and recognition of epidemiological links among cases. In 2011, the incidence rate of IMD was 0.73 per 100,000 in European Union (EU) countries, as reported by Marcus et al. in their report on a recent cluster of serogroup C *Neisseria meningitidis* (MenC) in MSM in this edition of *Eurosurveillance* [1], in the United States (US), it was 0.25 per 100,000 (an all-time low). While outbreaks of IMD in the US and EU are rare, they can be difficult to control, particularly when the primary risk factor is membership in a social network rather than an organisation or institution [2]. In September 2012, a slowly evolving outbreak of IMD was recognised among men who have sex with men (MSM) in New York City (NYC). The NYC Department of Health and Mental Hygiene (DOHMH), in collaboration with community providers, implemented a meningococcal vaccine campaign to prevent further illness and death [3]. IMD cases in MSM have recently also been recognised in Germany, France, and Belgium. In response to these clusters the European Centre for Disease Prevention and Control, with input from leading experts, composed and distributed a rapid risk assessment to help guide medical and public health authorities [4]. The report by Marcus et al. in this issue summarises the German cluster and highlights important issues regarding local and international IMD control [1].

The first reported outbreak of IMD in MSM occurred in Toronto in 2001 [5]. Subsequently, IMD outbreaks in the MSM community have occurred in Chicago in 2003 [6] and NYC in 2012 and 2013 [3]. All three outbreaks were determined to have been caused by MenC, multilocus sequence typing (MLST) sequence type 11 (ST-11), a common invasive strain of *Neisseria meningitidis*, and prompted public health officials to offer vaccination to people at risk. The IMD strain responsible for the cases in Germany also belongs to the MenC, ST-11 clonal complex, but direct comparisons to the Canadian and US strains have not yet been completed. No links to international travel have been identified between the European and US cases.

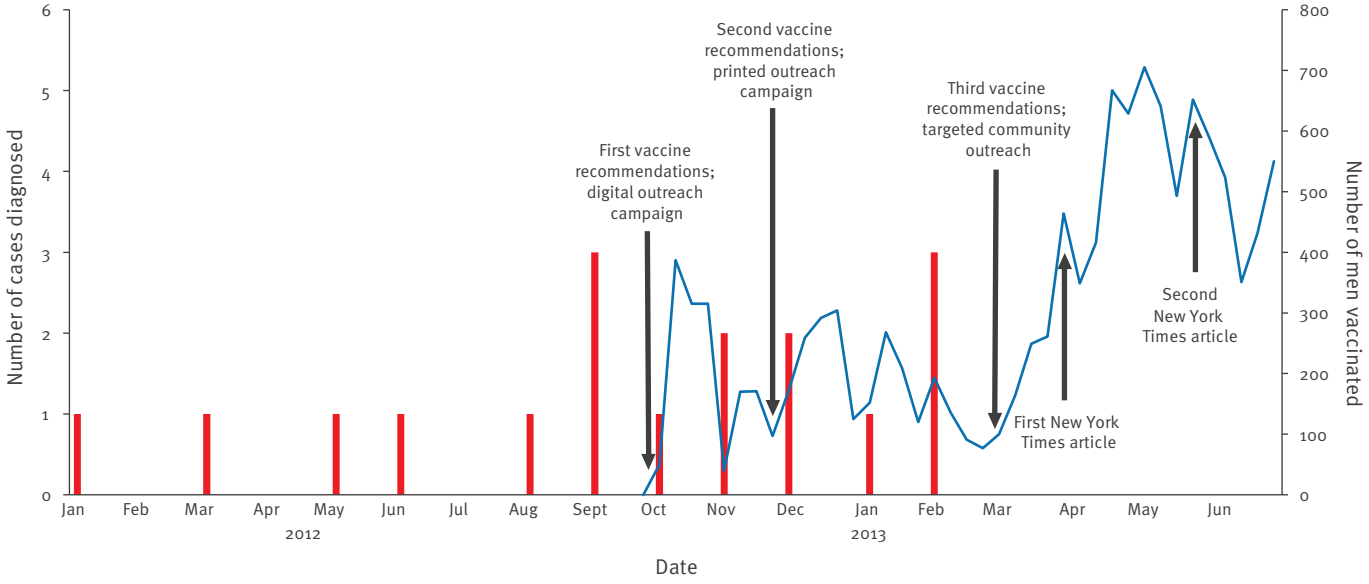
Although sexual partners are often elicited during IMD contact investigations, sexual orientation had not previously been incorporated into standardised questionnaires in the US or Europe. MSM may be at higher risk of IMD due to an increased frequency of known risk factors, such as bar patronage and smoking [7-9] or through previously unrecognised risk behaviours. Smoking behaviour in NYC among MSM aged 18 to 64 years, however, was not significantly different than among men who have sex only with women (for the years 2009–11: 24% versus 21%, p=0.4) [10]. While bar patronage was reported by Marcus et al. in the German cluster, only one of 17 recent MSM cases in NYC reported that they used bars to meet sexual partners. Drug use has appeared infrequently in the literature, but was reported by half of the MSM outbreak cases since 2012 in NYC (crystal methamphetamine, marijuana, cocaine). Whether these factors act through the sharing of cigarettes or drug paraphernalia, direct mucosal damage facilitating bacterial invasion, or via alteration of immune function is not currently known.

The relatively infrequent identification of this particular MenC strain in non-MSM is likely to represent circulation limited to the MSM social network. However, a unique mechanism of transmission or a susceptibility specific to MSM is possible. In the period August 2010 through June 2013, for those cases for whom pulsed-field gel electrophoresis was performed, only two of 16 MenC strain matches to the outbreak strain were not MSM, whereas only one of 15 cases in MSM was due an unrelated MenC strain. NYC is conducting a case–control study of IMD in MSM and performing full genome sequencing of MenC isolates from the past decade to better answer these questions.

The US Centers for Disease Control and Prevention (US CDC) and the German Standing Committee on Vaccination recommend using vaccine to control IMD

Figure 1

Invasive meningococcal disease among men who have sex with men and estimated vaccine doses, New York City, January 2012–June 2013



Shown are the number of individuals receiving the first dose of meningococcal vaccine. Numbers include only those public and private vaccine providers who agreed to provide weekly dose administration data and reporting is likely to be incomplete.

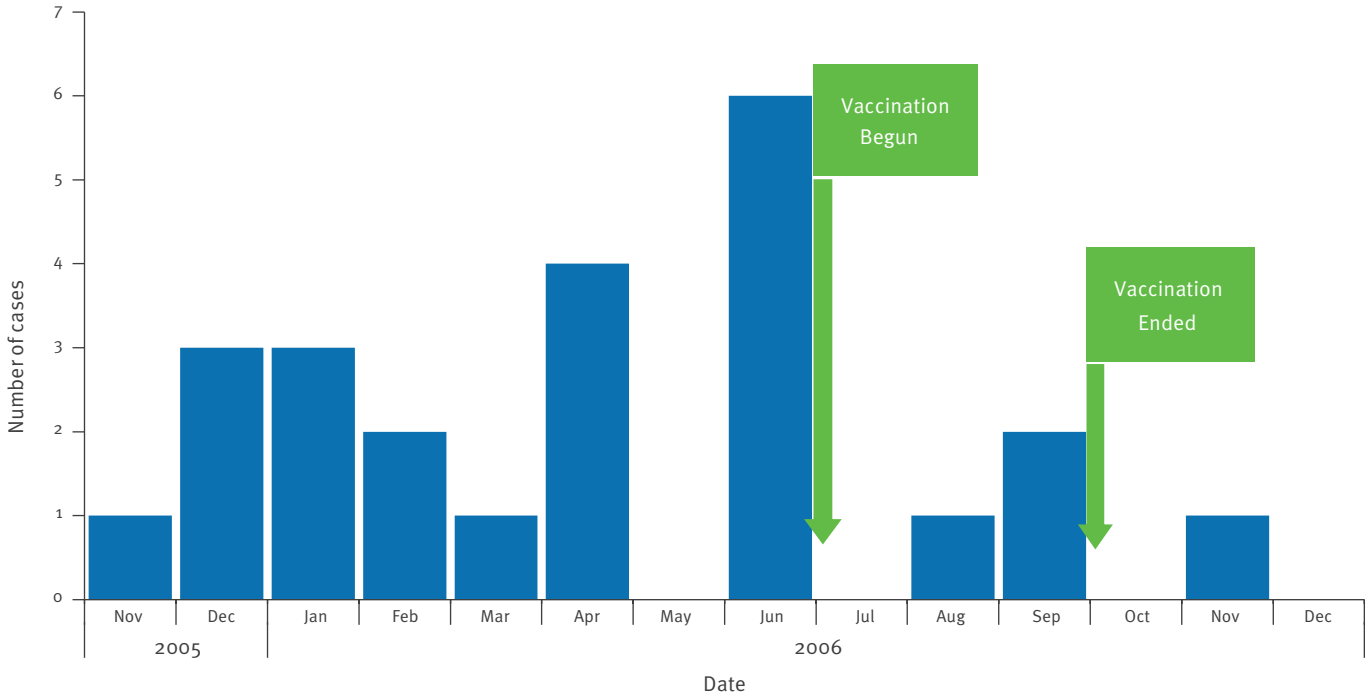
outbreaks when the attack rate exceeds a threshold suggestive of ongoing transmission [11-13]. While this recommendation can be readily applied to institutional outbreaks in which the population at risk is easily defined, it is much more difficult to apply to outbreaks in which the only common link is a social network or risk behaviours. In 2005 and 2006, an outbreak of IMD MenC, ST-11, occurred among recovering and current drug users and their close contacts in NYC [14]. The outbreak extended over many months without meeting strict US CDC criteria. The DOHMH responded by administering vaccine to 2,700 persons in affected neighbourhoods, utilising locations such as needle exchange sites, methadone clinics, and drug treatment centres [14].

There are many challenges to controlling IMD outbreaks. Contact tracing and antibiotic prophylaxis are the mainstays of public health practice for individual IMD cases. Cases are often unwilling or unable, however, to disclose sexual and drug using contacts. In NYC we have met with good success through the use of serial patient and family interviews and by utilising staff with experience in obtaining sensitive personal information. Vaccination is an attractive option for MenC IMD clusters; however, identifying the population at risk is challenging in community outbreaks. Vaccine campaigns consume substantial human and financial resources and at this time there is no evidence that a single dose of the vaccines available in the US confers either herd or long lasting immunity (personnel communication: Amanda Cohn, US CDC, October 2011). The current NYC vaccine campaign has cost over 1 million US dollars and has not yet ended. Narrowing the vaccine recommendations to target individuals at highest risk must be balanced against the difficulties of reaching cloistered populations and assuring adequate vaccine access for the under-insured.

A common link among MSM IMD cases in NYC has been use of the Internet and smartphone applications to meet sexual partners. The use of social media has recently emerged as a new method for communicating with individuals potentially exposed to an IMD case [15]. The DOHMH used email messages and mobile phone and online advertisements to advise the MSM community about the outbreak and need for vaccination. Interestingly, conventional media (major newspapers, network television) coverage sparked the largest uptakes in vaccine administration (Figure 1). In the Toronto and Chicago MSM outbreaks [5,6], vaccination campaigns coincided with the apparent cessation of IMD cases in the affected communities (Figures 1 and 2). We do not have definitive evidence that the vaccine campaigns halted these outbreaks. Nevertheless, it is plausible that vaccination campaigns reduce illness and death when focused on persons at highest risk. Defining that risk when the outbreak is occurring in social networks remains an important challenge for public health officials. Of note, France has issued vaccine recommendations for its MSM community based

Figure 2

Epidemic curve of invasive meningococcal disease cases among current and former drug users and their close contacts, New York City, 2005–06 (n=24)



on the recent European clusters [16], and this is under consideration in Germany [1].

IMD outbreaks among MSM communities in North America and Europe over the past 13 years highlight the common problems faced by public health officials. While the spread of IMD from the NYC MSM community to MSM communities in Europe has not been proven, the timing of cases is suspicious and emphasises the borderless nature of infectious diseases in the 21st century. International spread of IMD is not a new phenomenon [17]. After the Hajj pilgrimage in 2000, a serogroup W135 strain resulted in IMD cases in 16 countries [18]. The recent cases among MSM on both sides of the Atlantic emphasise the need for constant vigilance in the assessment of risk groups for IMD, and compel public health agencies to add questions about drug use and sexual practices to their routine investigations.

There is also a need to establish relationships and protocols for the sharing of epidemiologic data and microbiological specimens across continents and oceans, as exists for food-borne pathogens [19]. In light of the potential for similar outbreaks, routine vaccination of MSM, as well as persons infected with human immunodeficiency virus (HIV), have become important policy questions [20]. We are working to determine the cost-effectiveness of such a policy and as more is understood about the transmission and behavioural risk factors, how vaccination can be optimised to decrease the morbidity and mortality associated with IMD. Clinicians should keep IMD in their differential diagnosis when evaluating febrile MSM and public health officials are encouraged to inquire about the sexual partners and social networks of reported IMD cases.

References

1. Marcus U, Vogel U, Schubert A, Claus H, Baetzing-Feigenbaum J, Hellenbrand W, Wichmann O. A cluster of invasive meningococcal disease in young men who have sex with men in Berlin, October 2012 to May 2013. Euro Surveill. 2013;18(28):pii=20523. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20523
2. Brooks R, Woods CW, Benjamin DK, Rosenstein NE. Increased Case-Fatality Rate Associated with Outbreaks of Neisseria meningitidis Infection, Compared with Sporadic Meningococcal Disease, in the Untied States, 1994-2002. Clin Infect Dis. 2006;43(1):49-54. http://dx.doi.org/10.1086/504804. PMid:16758417.
3. Centers for Disease Control and Prevention (CDC). Notes from the field: serogroup C invasive meningococcal disease among men who have sex with men - New York City, 2010-2012. MMWR Morb Mortal Wkly Rep. 2013;61(51-42):1048.
4. European Centre for Disease Prevention and Control (ECDC). Invasive meningococcal disease among men who have sex with men. Rapid risk assessment. Stockholm: ECDC; 3 July 2013. Available from: http://ecdc.europa.eu/en/publications/Publications/rapid-risk-assessment-invasive-meningococcal-disease-among-MSM.pdf
5. Tsang RS, Kiefer L, Law DK, Stoltz J, Shahin R, Brown S, et al. Outbreak of serogroup C meningococcal disease caused by a variant of Neisseria meningitidis serotype 2a ET-15 in a community of men who have sex with men. J Clin Microbiol. 2003;41(9):4411-4. http://dx.doi.org/10.1128/JCM.41.9.4411-4414.2003. PMid:12958279. PMCid:PMC193786.
6. Schmink S, Watson JT, Coulson GB, Jones RC, Diaz, PS, Mayer LM, et al. Molecular Epidemiology of Neisseria menigitidis Isolates from an Outbreak of Meningococcal Disease among Men Who Have Sex With Men, Chicago Illinois, 2003. J Clin Microbiol. 2007;45(11): 3768-70. http://dx.doi.org/10.1128/JCM.01190-07. PMid:17728467. PMCid:PMC2168499.
7. Cookson ST, Corrales JL, Lotero JO, Regueira M, Binsztein N, Reeves MW, et al. Disco fever: epidemic meningococcal disease in northeastern Argentina associated with disco patronage. J Infect Dis. 1998;178(1):266–9. http://dx.doi.org/10.1086/517450. PMid:9652452.
8. Imrey PB, Jackson LA, Ludwinski PH, England AC 3rd, Fella GA, Fox BC, et al. Outbreak of serogroup C meningococcal disease associated with campus bar patronage. Am J Epidemiol. 1996;143(6):624-30. http://dx.doi.org/10.1093/oxfordjournals.aje.a008792 PMid:8610679.
9. Fischer M, Hedberg K, Cardosi P, Plikaytis BD, Hoesly FC, Steingart KR,et al. Tobacco smoke as a risk factor for meningococcal disease. Pediatr Infect Dis J. 1997;16(10):979-83. http://dx.doi.org/10.1097/00006454-199710000-00015. PMid:9380476.
10. New York City Department of Health and Mental Hygiene (DOHMH). Community Health Survey 2009-2011 combined. Custom data analysis by Bureau of Epidemiology Services, New York: DOHMH; 5 July 2013.
11. Control and prevention of serogroup C meningococcal disease: evaluation and management of suspected outbreaks: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep. 1997;46(RR-5):13-21. PMid:9048847
12. Cohn AC, MacNeil JR, Clark TA, Ortega-Sanchez IR, Briere EZ, Meissner HC, et al. Prevention and control of meningococcal disease: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep. 2013;62(RR-2):1-28. PMid:23515099.
13. German Standing Committee on Vaccination. Empfehlungen der Ständigen Impfkommission (STIKO) am Robert Koch-Institut. [Recommendations of the Standing Committee on Vaccination (STIKO) at the Robert Koch Institute]. Epidemiol Bull. 2012;30:283-310. German. Available from: http://www.rki.de/EN/Content/Prevention/Vaccination/recommandations/STIKO_Recommendations_2012_en.pdf?__blob=publicationFile)
14. Weiss D, Stern E, Zimmerman C, Bregman B, Yeung A, Das D, et al. Epidemiologic investigation and targeted vaccination initiative in response to an outbreak of meningococcal disease among illicit drug users in Brooklyn, New York. Clin Infect Dis. 2009;48(7):894-901. http://dx.doi.org/10.1086/597257. PMid:19231975.
15. Gounder P, DelRosso P, Adelson S, Rivera C, Middleton K, Weiss D. Using the Internet to trace Contacts of a Fatal Meningococcemia Case—New York City, 2010. J Public Health Manag Pract. 2012;18(4):379-81. PMid:22635194.
16. Haut Conseil de la Santé Publique. Avis relatif au recommandations de vaccination contre le méningocoque C, notamment chez les hommes ayant des relations sexuelles avec d'autres hommes. [Opinion on the recommendations for vaccination against meningococcus C, particularly among men who have sex with men. Paris: Haut Conseil de la Santé Publique; 3 Jul 2013. Available from: http://www.hcsp.fr/Explore.cgi/avisrapportsdomaine?clefr=356
17. Memish ZA. Meningococcal Disease and Travel. Clin Infect Dis. 2002;34(1):84-90. http://dx.doi.org/10.1086/323403. PMid:11731951.
18. Mayer L, Reeves M, Al-Hamdan N, Sacchi CT, Taha MK, Ajello GW, et al. Outbreak of W135 meningococcal disease in 2000: not emergence of a new W135 strain but clonal expansion within electrophoretic type-37 complex. J Infect Dis. 2002;185(11):1596-605. http://dx.doi.org/10.1086/340414. PMid:12023765.
19. Swaminathan B, Gerner-Smidt P, Ng LK, Lukinmaa S, Kam KM, Rolando S, et al. Building PulseNet International: an interconnected system of laboratory networks to facilitate timely public health recognition and response to foodborne disease outbreaks and emerging foodborne diseases. Foodborne Pathog Dis. 2006. Spring;3(1):36-50.
20. Simon MS, Weiss D, Gulick RM. Invasive Meningococcal Disease in Men Who Have Sex With Men. Ann Intern Med. 2013 June 17. http://dx.doi.org/10.7326/0003-4819-159-4-201308200-00674 PMid:23778867

A cluster of invasive meningococcal disease in young men who have sex with men in Berlin, October 2012 to May 2013

U Marcus (marcusu@rki.de)[1], U Vogel[2], A Schubert[3], H Claus[2], J Baetzing-Feigenbaum[3], W Hellenbrand[1], O Wichmann[1]
1. Department for Infectious Disease Epidemiology, Robert Koch Institute, Berlin, Germany
2. University of Würzburg, Institute for Hygiene and Microbiology and National Reference Laboratory for Meningococci, Würzburg, Germany
3. Infectious Disease Protection and Epidemiology Unit, State Office for Health and Social Affairs (LAGeSo), Federal State of Berlin, Berlin, Germany

Citation style for this article:
Marcus U, Vogel U, Schubert A, Claus H, Baetzing-Feigenbaum J, Hellenbrand W, Wichmann O. A cluster of invasive meningococcal disease in young men who have sex with men in Berlin, October 2012 to May 2013. Euro Surveill. 2013;18(28):pii=20523. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20523

Article submitted on 08 July 2013 / published on 11 July 2013

Between October 2012 and May 2013, five cases of invasive meningococcal disease in young men who have sex with men (MSM) living in Berlin were notified to local health authorities in Germany. Three of the five cases died. All were caused by serogroup C variants with the finetype P1.5-1,10-8:F3-6. Awareness was increased through the use of community networks; an extension of the existing vaccination recommendation to all MSM is currently being considered.

As of 2 July 2013, 208 cases of invasive meningococcal disease (IMD) were reported in Germany since the beginning of the year, which is similar to previous years (2012: 189 cases; 2011: 224 cases). Among the cases reported from Berlin (n=17) were three cases of IMD due to MenC in young men who have sex with men (MSM).

IMD is a rare but serious disease caused by *Neisseria meningitidis*, a gram-negative, encapsulated diplococcus. The clinical spectrum of IMD is diverse and may vary from a mild febrile illness to septicaemia and/or meningitis, which may progress to fulminant disease, multi-organ failure and death within hours [1]. The proportion of patients who develop severe disease increases with age [2].

Twelve distinguishable serogroups have been described [3]. *N. meningitidis* serogroups B (MenB) and C (MenC) predominate in Germany and affect mainly young children and adolescents [4]. Annual IMD incidence in Germany was 0.45 per 100,000 in the years 2010 to 2012 (n=386, n=369, n=354) [5]. Among young adults aged 20–29 years, IMD incidence in 2010 to 2012 was 0.65 per 100,000. Case fatality in 2012 was 9.3% and was highest in patients with MenC (13%) [5].

In 2006, the German Standing Committee on Vaccination (STIKO) recommended routine MenC vaccination in the second year of life. Catch-up vaccination for all children and adolescents under the age of 18 years on an individual basis is recommended, but a catch-up campaign was not undertaken. In 2010, MenC vaccination coverage at school entry ranged between 53% and 90% in the 16 German federal states [4]. In addition to routine childhood vaccination, STIKO recommends vaccination against serogroups A, C, W135 and Y for high-risk individuals independent of their age (i.e. patients with asplenia or immunocompromised individuals including those infected with human immunodeficiency virus (HIV)). However, meningococcal vaccine coverage among HIV patients in Germany is unknown. Vaccines are free of charge in Germany if officially recommended, but can otherwise be individually purchased if prescribed by a physician.

Description of cases

Case 1 was a man in his early 20s. He developed chills, fever and severe abdominal pain in early February, was hospitalised, and died during abdominal surgery within 10 hours after hospital admission. MenC was found in blood culture. The patient had visited several gay venues in Berlin in the days before falling ill. Cases 2 and 3 were both in their mid-20s. They visited a gay nightclub in May and spent the following night together. Two days later, Case 2 developed symptoms (fever, nausea, vomiting, irritability, stiff neck), was hospitalised, and treated in an intensive care unit. He survived, but suffers from irreversible brain damage. Case 3 developed symptoms a day later (irritability, fever, nausea), but did not seek medical care and died at home on the following day. An autopsy revealed death due to septic shock and disseminated intravascular coagulation. MenC was detected in cerebrospinal fluid.

Besides smoking (two cases) and attending gay bars, no other risk factors were reported. None of the three young men had been diagnosed with HIV, and none of

the three was vaccinated against MenC. While a common source or direct transmission between Cases 2 and 3 seems likely, no direct link between Case 1 and Cases 2 and 3 was found. No links to men who visited or lived in cities with concurrent or recent IMD outbreaks among MSM (e.g. Paris or New York) could be established [6,7].

Molecular typing

All three infections were due to *N. meningitidis* serogroup C: PorA-VR1 5-1, PorA-VR2 10-8: FetA F3-6 (C:P1.5-1,10-8:F3-6) and confirmed as sequence type (ST) 11. Analysis of position 640 of the *fumC* gene revealed that the strains belonged to a subclone of ST-11 designated electrophoretic type (ET) 15, which has caused a number of small outbreaks in Germany since 1998 [8]. The PorA and FetA variants are classically linked to ET-15 and the combination C:P1.5-1,10-8:F3-6 is observed frequently. Therefore, three further typing loci were included, i.e. *porB*, *fHbp*, and *penA*. All three isolates were identical also with regard to these markers: *porB* allele 2-2, *fHbp* allele 766, and *penA* allele 3. This finding supports the hypothesis of a local cluster and link between the three cases.

Retrospective epidemiology

In an analysis of data from the German disease notification system restricted to IMD cases aged 15 to 49 years occurring in the five largest metropolitan areas (i.e. Berlin, Hamburg, Munich, Cologne and Frankfurt), we identified in total n=15, n=11, n=14 and n=20 reported IMD cases in calendar weeks 1 to 27 for 2010, 2011, 2012 and 2013, respectively. In these five areas, the proportion of males with IMD in this specific age-group ranged between 40% and 54% in the period 2010 to 2012, whereas this proportion was 80% in 2013. In contrast, the sex distribution remained similar over the years when the analysis was not restricted to the five metropolitan areas (57%, 58%, 62% and 61% males, respectively).

When the local health authorities in Berlin became aware of this potential IMD outbreak among MSM, they investigated retrospectively if any of the other invasive MenC cases in young men notified in 2012–13 were MSM. Two additional cases were identified. One case was notified in February 2013, the other case in October 2012, both were in their late 20s. The latter case had developed signs of sepsis and died. Isolates from both cases had been characterised at the German Reference Laboratory for Meningococci as the same finetype PorA-VR1:5-1; PorA-VR2:10-8 and FetA:3-6. As the isolates were still available, further genetic typing was possible. Complementary typing has to date only been conducted for one isolate. The strain obtained from the case notified in February 2013 differed from the variant found in the series of Cases 1–3 with regard to the *penA* allele (*penA-2*) and the *fhbp* allele (new variant). This finding suggests that at least this case was caused by a highly related, yet distinct variant and that more than one strain was involved in this cluster of five cases.

Public health response

From October 2012 until the end of June 2013, the incidence of MenC IMD among the MSM community in Berlin was 6.3/100,000 based on five reported cases and an estimated number of 80,000 MSM in the community [9]. This is below the threshold of an epidemic situation (defined in Germany as ≥10 cases/100,000 in a given region within three months), but almost 10-fold higher than expected for young male adults in this age group for the entire year (0.65/100,000 with inclusion of all serogroups).

Infectious disease surveillance networks in Germany and healthcare professionals in Berlin were alerted to the detection of this IMD cluster among MSM, which will help to identify additional cases rapidly if they occur. The German AIDS support organisation Deutsche AIDS Hilfe has issued information on the cluster on their website as a first measure to inform the MSM community about symptoms of the disease and the existing recommendation to vaccinate HIV-positive individuals against IMD. According to STIKO, the existing meningococcal vaccination recommendation targeting risk groups (such as HIV patients) can be extended to other population subgroups by the responsible health authorities during regional outbreaks, taking into account the epidemiological and temporal associations between notified cases [10]. Since no HIV patients have been identified in the MSM cluster as of today, the state health authority in Berlin is currently considering the option of extending the existing vaccination recommendation to all MSM to prevent further cases.

Acknowledgements

We thank the local public health offices for collecting the relevant information and local laboratories for sending samples to the National Reference Center for Meningococci to conduct further molecular typing of strains.

Conflict of interest

None declared.

Authors' contributions

U. Marcus drafted the manuscript and participated in the outbreak investigation. U. Vogel supervised molecular typing of the bacterial isolates and contributed to the manuscript. A. Schubert provided data on the initial three-case cluster and initiated the retrospective case finding. H. Claus did the molecular typing of the isolates and contributed to the manuscript. J. Baetzing-Feigenbaum provided data on the cluster of five MSM from Berlin. W. Hellenbrand contributed to the manuscript. O. Wichmann coordinated the outbreak investigation and contributed to the manuscript.

References

1. Pace D, Pollard AJ. Meningococcal disease: clinical presentation and sequelae. Vaccine. 2012;30 Suppl 2:B3-9. http://dx.doi.org/10.1016/j.vaccine.2011.12.062. PMid:22607896.
2. Cohn AC, MacNeil JR, Harrison LH, Hatcher C, Theodore J, Schmidt M, et al. Changes in Neisseria meningitidis disease epidemiology in the United States, 1998-2007: implications for prevention of meningococcal disease. Clin Infect Dis. 2010;50(2):184-91. http://dx.doi.org/10.1086/649209. PMid:20001736.
3. Harrison OB, Claus H, Jiang Y, Bennett JS, Bratcher HB, Jolley KA, et al. Description and nomenclature of Neisseria meningitidis capsule locus. Emerg Infect Dis. 2013;19(4):566-73. http://dx.doi.org/10.3201/eid1904.111799. PMid:23628376. PMCid:PMC3647402.
4. Hellenbrand W, Elias J, Wichmann O, Dehnert M, Frosch M, Vogel U. Epidemiology of invasive meningococcal disease in Germany, 2002-2010, and impact of vaccination with meningococcal C conjugate vaccine. J Infect. 2013;66(1):48-56. http://dx.doi.org/10.1016/j.jinf.2012.09.008. PMid:23043893.
5. Robert Koch-Institut (RKI). Infektionsepidemiologisches Jahrbuch meldepflichtiger Krankheiten für 2012. [Annual epidemiological report on notifiable diseases for 2012] Berlin: RKI; 2013. German. Available from: http://www.rki.de/DE/Content/Infekt/Jahrbuch/jahrbuch_node.html
6. Simon MS, Weiss D, Gulick RM. Invasive Meningococcal Disease in Men Who Have Sex With Men. Ann Intern Med. 2013 Jun 17 http://dx.doi.org/10.7326/0003-4819-159-4-201308200-00674. PMid:23778867.
7. European Center for Disease Prevention and Control (ECDC). Rapid Risk Assessment: Invasive meningococcal disease among men who have sex with men. Stockholm: ECDC; 2013. Available from: http://www.ecdc.europa.eu/en/publications/Publications/rapid-risk-assessment-invasive-meningococcal-disease-among-MSM.pdf
8. Elias J, Vogel U. IS1301 fingerprint analysis of Neisseria meningitidis strains belonging to the ET-15 clone. J Clin Microbiol. 2007;45(1):159-67. http://dx.doi.org/10.1128/JCM.01322-06. PMid:17093016. PMCid:PMC1828961.
9. Marcus U, Schmidt AJ, Hamouda O, Bochow M: Estimating the regional distribution of men who have sex with men (MSM) based on Internet surveys. BMC Public Health. 2009;9:180. http://dx.doi.org/10.1186/1471-2458-9-180. PMid:19519888. PMCid:PMC2702383.
10. German Standing Committee on Vaccination. Empfehlungen der Ständigen Impfkommission (STIKO) am Robert Koch-Institut. [Recommendations of the Standing Committee on Vaccination (STIKO) at the Robert Koch Institute]. Epidemiol Bull. 2012;30:283-310. German. Available from: http://www.rki.de/EN/Content/Prevention/Vaccination/recommandations/STIKO_Recommendations_2012_en.pdf?__blob=publicationFile

Carbapenemase-producing *Enterobacteriaceae* in Europe: a survey among national experts from 39 countries, February 2013

C Glasner[1], B Albiger[2], G Buist[1], A Tambić Andrašević[3], R Canton[4,5], Y Carmeli[6], A W Friedrich[1], C G Giske[7,8], Y Glupczynski[9], M Gniadkowski[10], D M Livermore[11,12], P Nordmann[13,14], L Poirel[13,14], G M Rossolini[15], H Seifert[16], A Vatopoulos[17], T Walsh[12], N Woodford[18], T Donker[1], D L Monnet[2], H Grundmann (h.grundmann@umcg.nl)[1], the European Survey on Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) working group[19]

1. Department of Medical Microbiology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands
2. European Centre for Disease Prevention and Control, Stockholm, Sweden
3. Department of Clinical Microbiology, University Hospital for Infectious Diseases, Zagreb, Croatia
4. Servicio de Microbiología, Hospital Universitario Ramón y Cajal and Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain
5. Unidad de Resistencia a Antibióticos y Virulencia Bacteriana asociada al Consejo Superior de Investigaciones Científicas (CSIC), Madrid, Spain
6. Division of Epidemiology, Tel-Aviv Sourasky Medical Centre, Tel-Aviv, Israel
7. Clinical Microbiology, MTC - Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden
8. Swedish Institute for Communicable Disease Control, Solna, Sweden
9. National Reference Laboratory for Antibiotic Resistance Monitoring in Gram-negative Bacteria, CHU Mont Godinne, Université Catholique de Louvain, Yvoir, Belgium
10. Department of Molecular Microbiology, National Medicines Institute, Warsaw, Poland
11. Norwich Medical School, University of East Anglia, Norwich, United Kingdom
12. Section of Medical Microbiology IIB, School of Medical Sciences, Cardiff University, Heath Park Hospital, Cardiff, United Kingdom
13. INSERM U914 «Emerging Resistance to Antibiotics», Associated National Reference Center for Antibiotic Resistance, Faculté de Médecine et Université Paris-Sud, K. Bicêtre, France
14. Medical and Molecular Microbiology Unit, Department of Medicine, Faculty of Science, University of Fribourg, Switzerland
15. Dipartimento di Biotecnologie Mediche, Università di Siena, Siena; Dipartimento di Medicina Sperimentale e Clinica, Università di Firenze; SOD Microbiologia e Virologia, Azienda Ospedaliera-Universitaria Careggi, Firenze, Italy
16. Institute for Medical Microbiology, Immunology and Hygiene, Cologne University, Cologne, Germany
17. Department of Microbiology, National School of Public Health, Athens, Greece
18. Antimicrobial Resistance and Healthcare Associated Infections Reference Unit, Public Health England, London, United Kingdom
19. The EuSCAPE working group participants are listed at the end of the article

Citation style for this article:
Glasner C, Albiger B, Buist G, Tambić Andrašević A, Canton R, Carmeli Y, Friedrich AW, Giske CG, Glupczynski Y, Gniadkowski M, Livermore DM, Nordmann P, Poirel L, Rossolini GM, Seifert H, Vatopoulos A, Walsh T, Woodford N, Donker T, Monnet DL, Grundmann H, the European Survey on Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) working group. Carbapenemase-producing Enterobacteriaceae in Europe: a survey among national experts from 39 countries, February 2013 . Euro Surveill. 2013;18(28):pii=20525. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20525

Article submitted on 21 June 2013 / published on 11 July 2013

The spread of carbapenemase-producing *Enterobacteriaceae* (CPE) is a threat to healthcare delivery, although its extent differs substantially from country to country. In February 2013, national experts from 39 European countries were invited to self-assess the current epidemiological situation of CPE in their country. Information about national management of CPE was also reported. The results highlight the urgent need for a coordinated European effort on early diagnosis, active surveillance, and guidance on infection control measures.

The present report summarises the results from 39 European countries of a self-assessment of the epidemiological stage and the management of carbapenemase-producing *Enterobacteriaceae* (CPE) at national level.

Background

CPE are an emerging threat to healthcare and are frequently resistant to many other antibiotics than carbapenems [1,2] leaving few treatment options. The extent, to which healthcare systems have already been affected, however, differs substantially from country to country. Following a previous initiative, a group of European experts is implementing the European Survey on CPE (EuSCAPE) in an effort to update assessments of the nature and scale of CPE spread in Europe [3]. The current programme receives financial support from the European Centre for Disease Prevention and Control (ECDC). The aim of this study is to obtain a more accurate and timely estimate of CPE prevalence in European countries and to support reference laboratory-capacity building to prevent and control the spread of CPE in Europe.

Development of a questionnaire and collection of information

Table 1

Description of the epidemiological stages of carbapenemase-producing *Enterobacteriaceae* (CPE)

Epidemiological scale	Description	Stage
No cases reported	No cases reported	0
Sporadic occurence	Single cases, epidemiologically unrelated	1
Single hospital outbreak	Outbreak defined as two or more epidemiologically related cases in a single institution	2a
Sporadic hospital outbreaks	Unrelated hospital outbreaks with independent, i.e. epidemiologically unrelated introduction or different strains, no autochthonous inter-institutional transmission reported	2b
Regional spread	More than one epidemiologically related outbreak confined to hospitals that are part of a regional referral network, suggestive of regional autochthonous inter-institutional transmission	3
Inter-regional spread	Multiple epidemiologically related outbreaks occurring in different health districts, suggesting inter-regional autochthonous inter-institutional transmission	4
Endemic situation	Most hospitals in a country are repeatedly seeing cases admitted from autochthonous sources	5

The table was reproduced from reference [3].

A Scientific Advisory Board of European experts in the field of carbapenemase-producing bacteria was invited to provide scientific advice in support of the EuSCAPE programme management team. A questionnaire was devised and modified from a 'field-tested' version used during previous similar surveys [3]. The questionnaire was divided into two sections. The first section (13 questions) explored the experts' knowledge and awareness of the current occurrence of CPE according to a previously-established epidemiological staging system [1,3]. In brief, the system captures seven consecutive stages in the national spread of these organisms. The seven stages are described in Table 1.

The second section (22 questions) collected information about existing requirements, structures and guidance documents for reporting, surveillance, use of reference laboratory services and infection control for CPE. The questionnaire is available from the corresponding author.

In each of the 39 European countries (i.e. 27 European Union (EU) Member States, all European Economic Area (EEA)/ European Free Trade Association (ETFA) countries except Lichtenstein, and all EU enlargement countries, as well as Israel), a national expert (NE) with acknowledged laboratory and/or epidemiological experience was identified (for the United Kingdom two NEs participate in this questionnaire survey). The NEs were chosen among European Antimicrobial Resistance Surveillance Network (EARS-Net) contact points, experts from national reference diagnostic laboratories and ECDC-coordinating competent bodies. The list of NEs was validated by ECDC and represents the EuSCAPE Working Group. The NEs were invited to answer the questionnaire online (http://SurveyMonkey.net, SurveyMonkey Corporation, Portland, USA).

Answers from the NEs were compiled and analysed. When necessary, NEs were contacted by e-mail or telephone for clarification, and corrections were made accordingly. The epidemiological stage of some countries was considered as uncertain when (i) the NE reported a lack of awareness about the current epidemiology of CPE in their country, (ii) the answer of the NE indicated considerable underdetection and underreporting of CPE in their country, (iii) the comments made by the NE by e-mail or telephone indicated uncertainty and/or (iv) when frequent introductions into other countries have been described but the NE could not independently support this observation by own sources. In the maps (Figure), this uncertainty was indicated by displaying the respective country as hatched.

Results

All NEs completed the online questionnaire. Thirty-seven NEs declared that they were aware of the current epidemiology of CPE in their country and all rated the occurrence and spread of CPE in their country using the previously established epidemiological staging system (Figure and Table 1). Nevertheless, only 26 NEs could self-assess their current situation with certainty.

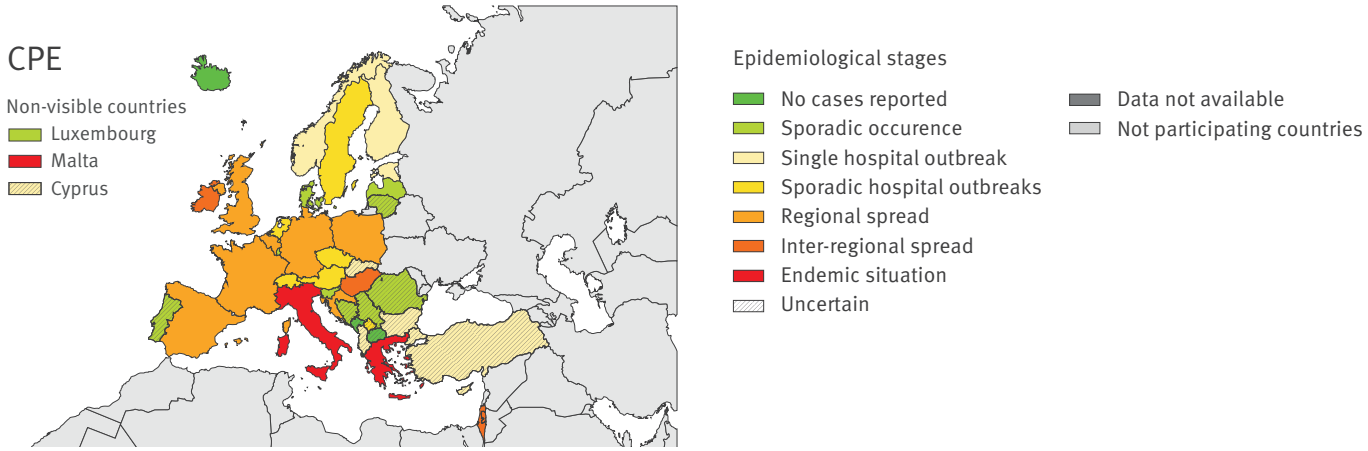
Three countries (Iceland, Montenegro and the former Yugoslav Republic of Macedonia) reported no cases of CPE in their country. Sporadic cases, single or sporadic hospital outbreaks were reported by NEs from 22 countries. For 11 countries, regional or national spread was reported, whereas for three countries (Greece, Italy and Malta) NEs reported that CPE are regularly isolated from patients in most hospitals, corresponding to the endemic stage (Table 2*).

Among the 31 countries that participated in both the 2010 and 2013 assessments, 17 reported a higher stage by 2013; likewise, by 2013, the number of countries with regional or inter-regional spread or an endemic situation increased from seven to 13 (Table 2*). Some countries expressed concerns that underdetection or

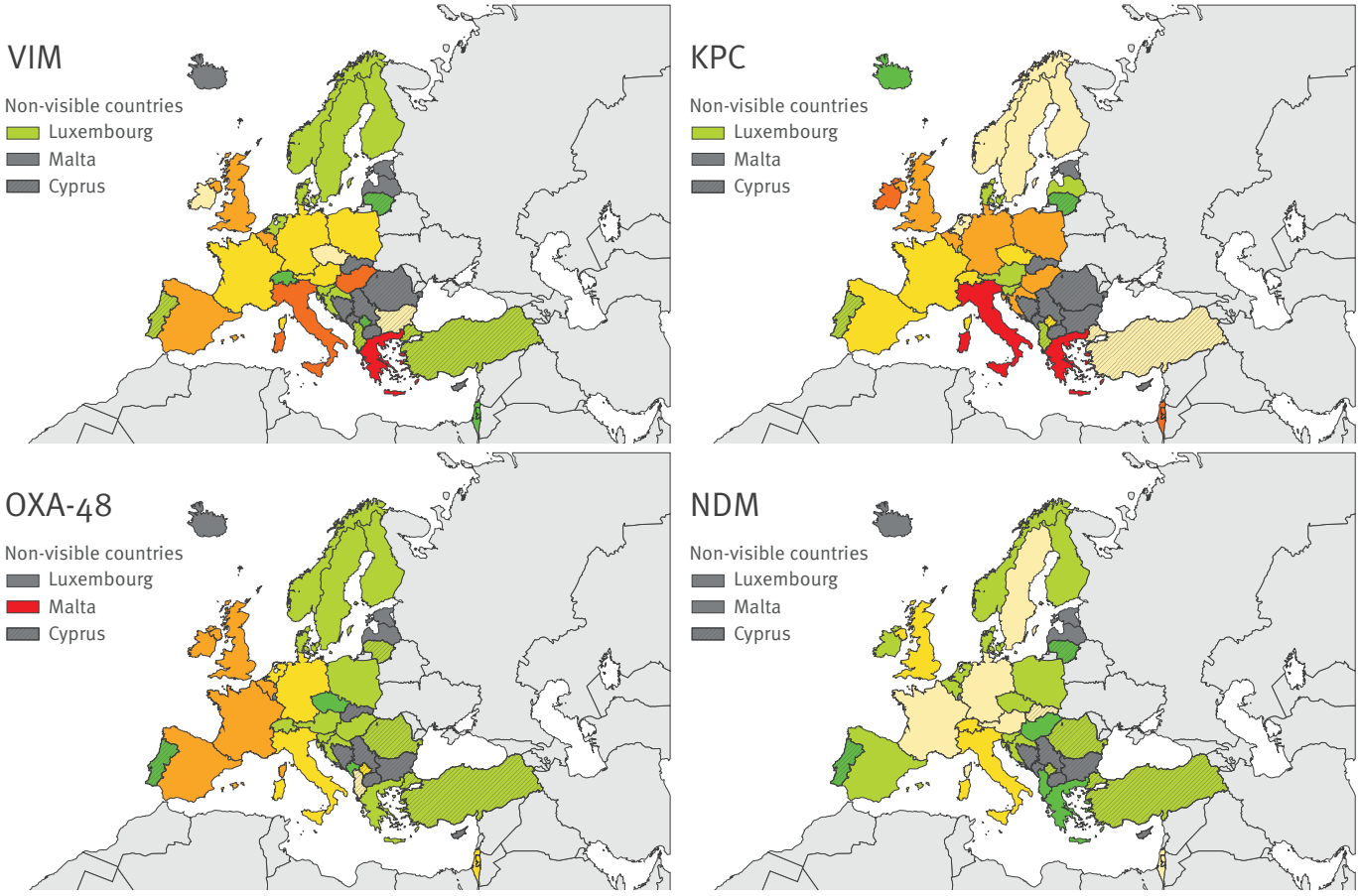
Figure

Occurrence of carbapenemase-producing *Enterobacteriaceae* (CPE) in 39 European countries based on self-assessment by respective national experts, 2013

A Overall European situation regarding CPE using an epidemiological scale of nationwide expansion



B Geographic distribution of CPE by resistance mechanism using the same epidemiological scale



KPC: *Klebsiella pneumoniae* carbapenemase-producing *Enterobacteriaceae*; NDM New Delhi metallo-beta-lactamase; OXA-48: carbapenem-hydrolysing oxacillinase-48; VIM: Verona integron-encoded metallo-beta-lactamase.

More details on the epidemiological stages are given in the manuscript Table 1.

In some countries, the epidemiological stage might not represent the true extent of the spread of CPE as it is a subjective judgment by national experts. Uncertainty about the epidemiological stage of a country is indicated by hatching. Results presented here reflect the uncertainty at the time of the survey. For Portugal, case notification and submission of isolates became mandatory on 21 February 2013.

Table 2

Comparison of epidemiological stages of carbapenemase-producing *Enterobacteriaceae* (CPE) in 39 European countries, 2010, 2012 and 2013

Country	Epidemiological stage for spread of CPE			Direction of change (2010 compared to 2013)[d]
	Grundmann et al., 2010[a]	Canton et al., 2012[b]	2013[c]	
Albania	NA	NA	2a	NA
Austria	0	1	2b	↑
Belgium	2b	3	3	↑
Bosnia and Herzegovina	1	1	1	→
Bulgaria	0	NA	2a	↑
Croatia	1	1	3	↑
Cyprus	2a	NA	2a	→
Czech Republic	1	1	2b	↑
Denmark	1	1	1	→
Estonia	0	NA	2a	↑
Finland	1	1	2a	↑
France	3	4	3	–[e]
Germany	3	3	3	→
Greece	5	5	5	→
Hungary	3	2a	4	↑
Iceland	0	0	0	→
Ireland	1	1	4	↑
Israel	5	5	4	↓
Italy	4	5	5	↑
Kosovo[f]	NA	1	3	NA
Latvia	1	NA	1	→
Lithuania	1	NA	1	→
Luxembourg	NA	1	1	NA
Malta	1	NA	5	↑
Montenegro	NA	1	0	–[g]
Netherlands	2a	2b	2b	↑
Norway	2a	2a	2a	→
Poland	4	4	3	–[e]
Portugal	1	1	1	→
Romania	1	1	1	→
Serbia	NA	1	1	NA
Slovakia	NA	NA	2b	NA
Slovenia	0	1	1	↑
Spain	2b	2b	3	↑
Sweden	2a	2a	2b	↑
Switzerland	1	1	2b	↑
The Former Yugoslav Republic of Macedonia	NA	NA	0	NA
Turkey	NA	4	2a	–[g]
United Kingdom	2b	3	3	↑

NA: not available.

The epidemiological staging system, developed in 2010, is based on seven levels [3]. Stage 0: no case reported; stage 1: sporadic occurrence whereby only single cases are reported; stage 2a: single hospital outbreak reported whereby an outbreak is defined as two or more epidemiologically-associated cases with indistinguishable geno- or phenotype; stage 2b: sporadic hospital outbreaks reported whereby more than one hospital outbreak is reported but all outbreaks are epidemiologically unrelated or caused by different clones (no autochthonous inter-institutional transmission); stage 3: regional spread whereby more than one epidemiologically-related hospital outbreak is reported, but confined to the same region or health district (regional autochthonous inter-institutional transmission); stage 4: inter-regional spread whereby multiple epidemiologically-related hospital outbreaks are reported from different regions or health districts (inter-regional autochthonous inter-institutional transmission); and stage 5: endemic situation whereby most hospitals in a country are constantly seeing cases admitted from autochthonous sources.

The epidemiological stage of a country may not reflect the true extent of the spread of CPE, as it is based on the subjective judgment of the responding national expert in 2010 and 2013 and the opinion of the authors of a review in 2012.

Some of the countries were not included in the 2010 survey and/or the 2012 review and their epidemiological stage is consequently indicated as 'not available' (NA).

[a] The results were based on data obtained through a European-wide consultation during a workshop at the Netherlands's National Institute for Public Health and the Environment (RIVM) on 29 and 30 April 2010 [3].

[b] The results were based on the subjective analyses of the literature available at the time of the publication [1].

[c] This online survey (February 2013).

[d] ↑ = increase in the epidemiological stage, ↓ = decrease in the epidemiological stage and → = unchanged epidemiological stage. A dash indicates that there are discrepancies between the results of the 2012 review and the 2013 survey, whereby no direction of change can be given.

[e] For France and Poland, discrepancies between results from the 2012 review and the 2013 survey are probably due to the subjective assessment by different experts.

[f] This designation is without prejudice to positions on status, and is in line with United Nations Security Council resolution 1244/99 and the International Court of Justice Opinion on the Kosovo declaration of independence.

[g] For Montenegro and Turkey, discrepancies between results from the 2012 review and the 2013 survey underline the uncertainty of stage designation for these countries.

underreporting, or both, could affect the certainty of the stage of their countries (Figure).

Thirty-three of the NEs indicated that *Klebsiella pneumoniae* was the most frequent *Enterobacteriaceae* species to produce carbapenemases in their country. Overall, *K. pneumoniae* carbapenemase-producing *Enterobacteriaceae* (KPC) have attained the widest distribution, whereas strains with New Delhi metallo (NDM)-beta-lactamase – although responsible for occasional hospital outbreaks in few countries – have not reached such a wide distribution in European countries (Figure).

Table 3* displays the level of national management of CPE, based on existing surveillance, reference systems, and guidance in the 39 countries. Thirty and 29 of 39 countries reported having a dedicated surveillance system for CPE and a dedicated reference laboratory for CPE, respectively. Twenty-three reported having a system to notify CPE cases to health authorities, mostly on a mandatory basis. Only 22 countries reported having national recommendations or guidelines on infection control measures to prevent the spread of CPE; one country reported having such recommendation or guideline in preparation.

Countries that were uncertain about their epidemiological stages had on average 1.9 national management documents regulating surveillance and response structures. In contrast, those who were more certain about their epidemiological stages had on average 4.7 (p-value < 0.001; Wilcoxon Rank Sum Test).

Discussion

The results of this online survey, performed in February 2013, show that, based on the knowledge and judgment of NEs, CPE are continuing to spread in Europe. Although most countries reported only single hospital outbreaks, the epidemiological situation has deteriorated over the past three years. Among the 31 countries that participated in both 2010 and 2013 assessments, 17 countries were upgraded to a higher epidemiological stage (Table 2). Three countries that reported sporadic occurrence or single hospital outbreaks of CPE in 2010 are now witnessing regional or inter-regional spread, or even an endemic situation. Malta moved from having sporadic cases to an endemic situation, although by nature of its small size, the intermediate epidemiological stages have little relevance. The influx of injured refugees from Libya in 2011, is believed to have contributed to an increase in carbapenem-hydrolysing oxacillinase (OXA)-48-positive *Enterobacteriaceae* (M. Borg, personal communication, April 2013). In Italy, a sporadic occurrence of Verona integron-encoded metallo-beta-lactamase (VIM)-producing *Enterobacteriaceae* from 2008, accentuated by a single hospital outbreak, has been overtaken by the wide dissemination of KPC-positive *K. pneumoniae* strains to many healthcare institutions. [4-9]. The situation in Hungary has evolved in the opposite direction: in 2010, concern centred upon a single clone of KPC-2-positive *K. pneumoniae* that had attained regional distribution, whereas VIM-4-positive strains were only reported sporadically, but have now spread nationwide [3,10]. Overall, KPC-positive *Enterobacteriaceae* still have the widest distribution among CPE in Europe, but rising numbers of OXA-48-positive isolates are reported, making OXA-48 the most frequently detected carbapenemase in Belgium, France and Malta. Despite the attention that NDM has received when associated with introductions from the Indian subcontinent, the current numbers of reports by European countries are still relatively modest compared to the other carbapenemases [11]. The United Kingdom, however, continues to report more NDM-positive isolates than most other European countries [3,12].

The NEs completed the questionnaire to the best of their knowledge, but these were subjective assessments that may have underestimated the true extent of the spread of CPE. Underdetection and underreporting were pointed out by respondents in several countries, leading to uncertainty about the true epidemiological stage (Figure). In particular, this applied to countries from which introductions into other countries have been described but where NEs could not independently assess the extent of CPE spread. Underdetection and underreporting of CPE also coincided with weaker reference laboratory infrastructures and the absence of national recommendations for submission to national reference laboratories and for reporting to health authorities, thus suggesting that the true extent of CPE occurrence in Europe is still underestimated. At the same time, countries with strict screening policies and good surveillance are more likely to report advanced epidemiological stages also affecting the comparability of the assessment.

The keys to success in preventing the establishment of CPE are, firstly, early detection through good diagnostic practices, secondly, containment of spread through patient and contact screening as well as infection control measures. An increasing number of countries have reacted and implemented measures as indicated by the increasing availability of a recommendation or guideline on infection control measures to prevent the spread of CPE [12]. Still 17 countries surveyed lacked such guidance and the same number of countries lacked relevant guidance for submission of isolates to national reference laboratories [12]. The results of the present report underscore the urgent need for an upgrading of laboratory standards to enable active surveillance and preventive action. To this purpose, the EuSCAPE programme aims to build a laboratory-based network for CPE detection in Europe.

Table 3

National management of carbapenemase-producing *Enterobacteriaceae* (CPE) in 39 European countries, 2013*

Country	National system for surveillance	Officially nominated national reference laboratory	National recommendation or guideline for submitting isolates to national expert or reference laboratories	Agreed criteria or a policy for submitting isolates to national expert or reference laboratories[a]	National recommendation or obligation for reporting (notification) to health authorities	National recommendation or guideline on infection control measures
Albania						
Austria	●	●	●	●	●[b]	●
Belgium	●	●	●	●	●[b]	●
Bosnia and Herzegovina						
Bulgaria	●	●	●	●	●[c]	●
Croatia	●	●	●	●	●[c]	●
Cyprus	●					
Czech Republic	●	●	●	●	●[c]	●
Denmark	●	●	●	●		
Estonia	-[d]					
Finland	●	●	●	●	●[c]	
France	●	●	●	●	●[c]	●
Germany	●	●		●		●
Greece	●	●	●	●	●[c]	●[e]
Hungary	●	●	●	●	●[c]	●
Iceland	●	●	●	●	●[c]	●
Ireland	●	●	●	●	●[c]	●[e]
Israel	●	●			●[c]	●
Italy	●				●[c,f]	●
Kosovo[g]		●	●			●[e]
Latvia	-[d]	●			●[c]	
Lithuania	●	●		●		
Luxembourg	●	●		●	●[c]	
Malta	●	●	●	●		●[e]
Montenegro						
Norway	●	●	●	●	●[c]	●
Poland	●	●	●	●	●[c]	●
Portugal	●	●	●	●	●[c]	●
Romania	-[d]	●				
Serbia	●	●				
Slovakia	●	●			●[b]	
Slovenia			●	●		●
Spain	●	●	●	●	●[b]	
Sweden	●	●	●	●	●[c]	●
Switzerland	●			●		
The Former Yugoslav Republic of Macedonia	●	●			●[c]	
The Netherlands	●		●	●	●[b]	●
Turkey						
United Kingdom	●	●	●	●		●

In the table cells, a dot in signifies 'in place' and the absence of a dot signifies 'absent'.

[a] Agreed criteria or policy (including minimum inhibitory concentration (MIC) cut-off, species and resistance confirmation, epidemiological typing) to submit CPE isolates to a national reference laboratory.

[b] Voluntary notification to health authorities.

[c] Mandatory notification to health authorities.

[d] Country reporting carbapenem-resistant invasive isolates (*Klebsiella pneumoniae* and *Escherichia coli* to the European Antimicrobial Resistance Surveillance Network (EARS-Net)).

[e] Only in case of outbreaks.

[f] Only for bacteraemia cases.

[g] This designation is without prejudice to positions on status, and is in line with United Nations Security Council resolution 1244/99 and the International Court of Justice Opinion on the Kosovo declaration of independence.

The European Survey on Carbapenemase-Producing *Enterobacteriaceae* (EuSCAPE) working group (national experts)

Albania – Andi Koraqi; Austria – Petra Apfalter; Belgium – Youri Glupczynski; Bosnia and Herzegovia – Tatjana Marković; Bulgaria – Tanya Strateva; Croatia – Arjana Tambić Andrašević; Cyprus – Despo Pieridou-Bagatzouni; Czech Republic – Jaroslav Hrabak; Denmark – Anette M. Hammerum, Estonia – Marina Ivanova; Finland – Jari Jalava; France – Bruno Coignard; Germany – Martin Kaase; Greece – Alkis Vatopoulos; Hungary – Ákos Tóth; Iceland – Hordur Hardarson; Ireland – Teck Wee Boo; Israel – Yehuda Carmeli; Italy – Annalisa Pantosti; Kosovo – Lul Raka; Latvia – Arta Balode; Lithuania – Jolanta Miciuleviciene; Luxembourg – Monique Perrin-Weniger; Malta – Nina Nestorova; Montenegro – Gordana Mijović; The Netherlands – Henk Bijlmer; Norway – Ørjan Samuelsen; Poland – Dorota Żabicka; Portugal – Manuela Caniça; the former Yugoslav Republic of Macedonia – Ana Kaftandzieva; Romania – Maria Damian; Scotland – Camilla Wiuff; Serbia – Zora Jelesić; Slovakia – Milan Nikš; Slovenia – Mateja Pirš; Spain – Jesùs Oteo; Sweden – Christian G. Giske; Switzerland – Andrea Endimiani; Turkey – Deniz Gür; United Kingdom – Neil Woodford.

Acknowledgements

The European Survey on Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) is funded by ECDC through a specific framework contract (ECDC/2012/055) following an open call for tender (OJ/25/04/2012-PROC/2012/036). Switzerland does not receive ECDC funding, but contributes to the survey using resources from the National Surveillance Program 'ANRESIS' (www.anresis.ch) funded by the Federal Office of Public Health.

Conflict of interest

None declared.

Authors' contributions

Corinna Glasner, Barbara Albiger, Dominique Monnet, Hajo Grundmann: wrote the manuscript. Corinna Glasner, Barbara Albiger, Girbe Buist, Arjana Tambić Andrašević , Rafael Cantón, Yehuda Carmeli, Alexander W. Friedrich, Christian G. Giske, Youri Glupczynski, Marek Gniadkowski, David M. Livermore, Patrice Nordmann, Laurent Poirel, Gian Maria Rossolini, Harald Seifert, Alkiviadis Vatopoulos, Timothy Walsh, Neil Woodford, Dominique Monnet, Hajo Grundmann and the EuSCAPE working group: provided feedback, contributed with comments and reviewed the manuscript. Tjibbe Donker: provided technical assistance with the production of the maps. Corinna Glasner, Barbara Albiger, Girbe Buist, Arjana Tambić Andrašević, Rafael Cantón, Yehuda Carmeli, Alexander W. Friedrich, Christian G. Giske, Youri Glupczynski, Marek Gniadkowski, David M. Livermore, Patrice Nordmann, Laurent Poirel, Gian Maria Rossolini, Harald Seifert, Alkiviadis Vatopoulos, Timothy Walsh, Neil Woodford, Dominique L. Monnet, Hajo Grundmann: designed and reviewed the questionnaire survey. Corinna Glasner, Hajo Grundmann: supervised and coordinated the survey with the EuSCAPE working group in Europe. Corinna Glasner, Barbara Albiger, Dominique Monnet, Hajo Grundmann: performed the data analysis. The EuSCAPE working group: answered the survey and provided the country specific data.

* Authors' correction

The following corrections were made at the request of the authors on 15 August 2013: the numbers of the tables have been corrected throughout the text and corrections have been made in Table 3 for Ireland, Kosovo, Latvia and the Netherlands. On 24 November 2014, additional amendments to Table 3 were implemented for Bulgaria, and parts of the text describing this Table were modified accordingly.

References

1. Cantón R, Akóva M, Carmeli Y, Giske CG, Glupczynski Y, Gniadkowski M, et al. Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. Clin Microbiol Infect. 2012;18(5):413–31. http://dx.doi.org/10.1111/j.1469-0691.2012.03821.x. PMid:22507109.
2. Hawkey PM. The growing burden of antimicrobial resistance. J Antimicrob Chemother. 2008;62(Suppl 1):i1–i9. http://dx.doi.org/10.1093/jac/dkn241. PMid:18684701.
3. Grundmann H, Livermore DM, Giske CG, Cantón R, Rossolini GM, Campos J, et al. Carbapenem-non-susceptible Enterobacteriaceae in Europe: conclusions from a meeting of national experts. Euro Surveill. 2010;15(46):pii=19711. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19711. PMid:21144429.
4. Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis. 2011;17(10):1791–8. http://dx.doi.org/10.3201/eid1710.110655. PMid:22000347. PMCid:PMC3310682.
5. Walsh TR. Emerging carbapenemases: a global perspective. Int J Antimicrob Agents. 2010;36 Suppl 3:S8–14. http://dx.doi.org/10.1016/S0924-8579(10)70004-2
6. Agodi A, Voulgari E, Barchitta M, Politi L, Koumaki V, Spanakis N, et al. Containment of an outbreak of KPC-3-producing Klebsiella pneumoniae in Italy. J Clin Microbiol. 2011;49(11):3986–9. http://dx.doi.org/10.1128/JCM.01242-11. PMid:21900525. PMCid:PMC3209099.
7. Rossolini GM, Riccio ML, Cornaglia G, Pagani L, Lagatolla C, Selan L, et al. Carbapenem-resistant Pseudomonas aeruginosa with acquired bla(VIM) metallo-beta-lactamase determinants, Italy. Emerg Infect Dis. 2000;6(3):312–3. http://dx.doi.org/10.3201/eid0603.000314. PMid:10939846. PMCid:PMC2640878.
8. Rossolini GM, Luzzaro F, Migliavacca R, Mugnaioli C, Pini B, De Luca F, et al. First countrywide survey of acquired metallo-beta-lactamases in Gram-negative pathogens in Italy. Antimicrob Agents Chemother. 2008;52(11):4023–9. http://dx.doi.org/10.1128/AAC.00707-08. PMid:18809945. PMCid:PMC2573113.
9. Grundmann H, Aanensen DM, van den Wijngaard CC, Spratt BG, Harmsen D, Friedrich AW, et al. Geographic distribution of Staphylococcus aureus causing invasive infections in Europe: a molecular-epidemiological analysis. PLoS Med. 2010;7(1):e1000215. http://dx.doi.org/10.1371/journal.pmed.1000215. PMid:20084094. PMCid:PMC2796391.
10. Toth A, Damjanova I, Puskás E, Jánvári L, Farkas M, Dobák A, et al. Emergence of a colistin-resistant KPC-2-producing Klebsiella pneumoniae ST258 clone in Hungary. Eur J Clin Microbiol Infect Dis. 2010;29(7):765–9. http://dx.doi.org/10.1007/s10096-010-0921-3. PMid:20401676.
11. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis. 2010;10(9):597–602. http://dx.doi.org/10.1016/S1473-3099(10)70143-2
12. European Centre for Disease Prevention and Control (ECDC). Risk assessment on the spread of carbapenemase-producing Enterobacteriaceae (CPE) through patient transfer between healthcare facilities, with special emphasis on cross-border transfer. Stockholm:ECDC; 2011. Available from: http://ecdc.europa.eu/en/publications/Publications/110913_Risk_assessment_resistant_CPE.pdf

Prevalence of human immunodeficiency virus and hepatitis C virus among French prison inmates in 2010: a challenge for public health policy

C Semaille (c.semaille@invs.sante.fr)[1], Y Le Strat[1], E Chiron[1], K Chemlal[2], M A Valantin[2], P Serre[3,4], L Caté[5], C Barbier[5], M Jauffret-Roustide[1], the Prevacar Group[6]

1. Institut de Veille Sanitaire, Saint-Maurice, France
2. Centre Hospitalier de la Pitié Salpetrière, Paris, France
3. Unité de consultations et de soins ambulatoires, Centre Hospitalier le Mans, Le Mans, France
4. Associations des Professionnels de Santé Exerçant en Prison (APSEP), France
5. Direction Générale de la Santé, Paris, France
6. The members of the group are listed at the end of the article

Citation style for this article:
Semaille C, Le Strat Y, Chiron E, Chemlal K, Valantin MA, Serre P, Caté L, Barbier C, Jauffret-Roustide M, the Prevacar Group. Prevalence of human immunodeficiency virus and hepatitis C virus among French prison inmates in 2010: a challenge for public health policy. Euro Surveill. 2013;18(28):pii=20524. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20524

Article submitted on 31 August 2012 / published on 11 July 2013

We evaluated prevalence of human immunodeficiency virus (HIV) and hepatitis C virus (HCV) among prison inmates in France in 2010, in a cross-sectional single-day study based on a two-stage design. Sampling favoured larger establishments and included all types of prisons. Establishments were stratified by geographical region. Estimates were adjusted by post-stratification of the total population of inmates in France. From 60,975 inmates in all 188 prisons on the sampling day, 2,154 were selected from 27 prisons, and 1,876 questionnaires completed. HIV prevalence was estimated at 2.0% (95% confidence interval (CI): 0.9–4.2), 2.6% (95% CI: 0.7–8.8) in women and 2.0% (95% CI: 0.9–4.3) in men; 75% of inmates were receiving treatment for HIV. HCV prevalence was estimated at 4.8% (95% CI: 3.5–6.5) and was higher for women (11.8%; 95% CI: 8.5–16.1) than men (4.5%; 95% CI: 3.3–6.3). Almost half of HCV-infected inmates had chronic hepatitis C and 44% were receiving or had received treatment. HIV and HCV prevalence was six times higher than in the general population, and 2.5% of inmates had viraemic hepatitis C. The moment of incarceration provides an ideal opportunity for testing and treating, limiting spread of HCV and improving patients' prognosis.

Introduction

Infectious diseases are more prevalent in prison than in the general population, in particular human immunodeficiency virus (HIV) and hepatitis C. This is well documented in the international literature [1-5]. In France, foreign nationals represent 18% of admissions to French prisons, and more than half of these entrants originate from countries with a generalised HIV epidemic and from regions with high or medium hepatitis C virus (HCV) endemicity [6]. Since 1 January 2009, drug-trafficking offences have accounted for nearly 14% of convicted prisoners.

Published prevalence data are usually taken from studies conducted on small numbers of prisons or performed in single regions [7-14]. Previous HIV and HCV prevalence studies in French prisons were either conducted in a single region or did not contain information on the characteristics of the infected persons [15-20]. Implementing epidemiological studies in a prison environment presents more challenges than studies in the community, notably because of ethical considerations [21]. National data on HIV and HCV prevalence are however essential to implement prevention interventions and to improve screening and treatment for these two chronic conditions.

In this article, we report the results of a cross-sectional, single-day study based on two-stage sampling of prison establishments and inmates that was conducted in 2010 (Prévacar survey). Estimates of HIV and HCV infection prevalence were produced for the entire prison population, and by sex, age and continent of birth.

Methods

Target population

The eligible population for the survey comprised any individual aged over 18 and held in prison on the sampling day (15 May 2010) in metropolitan France and/or in French overseas departments (Antilles, French Guiana and Reunion Island). Prisoners on licence or parole on the sampling day were excluded from the survey.

Sample size calculation

The number of individuals for inclusion in the study was calculated to take into account the proportion of prisoners receiving opiate substitute treatments (estimated

at 10%), the absolute level of precision required in the estimates (2%), and the expected design effect produced by the two-stage sampling design employed. The necessary sample size was 1,300 subjects, and 2,154 people were randomly selected in the prison establishments.

Sampling design

The French national prison service has a complete listing of prison establishments in France, and an exhaustive national database of prison inmates which is updated several times per day. Prisoners in the national database are assigned a unique identification number.

To limit the number of prison establishments, we implemented a two-stage sampling design instead of using simple random sampling. Prison establishments and prisoners were selected at the first and second stage, respectively. At the first stage, the exhaustive list of prison establishments was stratified according to ten geographical regions in France, i.e. covering the nation's territory. Within each region, establishments were further stratified to take into account the type of establishment (short, medium or long sentences, female-only or male-only). Establishments were chosen using ordered systematic sampling with unequal probabilities, proportional to the number of prisoners. Establishments for women and in certain priority regions were deliberately oversampled. At the second stage prisoners were selected by simple random sampling using the unique identification number in the national database of people in French prisons on the sampling day.

Data collection

The study did not use biological testing. Instead, for each person sampled, an anonymous individual questionnaire was completed by the Prévacar researchers or by health professionals based in the prison establishments using information in medical records. Proposing HIV and hepatitis C testing for all prisoners on admission to prison is mandatory in France, and French guidelines recommend that this offer be repeated periodically during prison stays.

The questionnaire also collected information on modes of transmission, clinical stage, treatment, viral load, CD4 lymphocyte count, and hepatic fibrosis. Additional information concerning continent of birth, socioeconomic status and employment before imprisonment was collected from the national prisoner database and merged with the questionnaire data using the unique identification number.

Definition of cases

A prison inmate was considered to be HIV infected when their medical record contained one of the following items: a positive ELISA test for HIV infection or a positive Western blot or a CD4 lymphocyte count or HIV viral load. A prison inmate was considered to be HCV infected when their medical record contained one of the following elements: a positive ELISA test for HCV or a positive HCV RNA detection in the previous 12 months.

Ethical considerations

To preserve the anonymity of subjects, a random number (different from the unique prison identification number mentioned above) was assigned to each selected prisoner. Prior to the study, the 2,154 people randomly selected in the prison establishments were informed individually of their selection and information on the study was posted publicly. At this point, prisoner refusal to take part in the survey could be communicated verbally or by returning a reply coupon to the medical team. The survey was approved by an ethics committee (no. 909331) and conformed to the principles embodied in the Declaration of Helsinki.

Statistical analysis

Analyses took into account the weighted sample design, i.e. oversampling for size and type of establishment, and estimates were adjusted by post-stratification on the total prison population [22] (n=60,975 on the sampling day) using available prison data (including sex, age group, continent of birth and geographical prison region) recorded for all prisoners in the national prisoner database on the date of sampling. Post-stratification took into account the national distribution of inmates by type of establishment.

This means that even for persons whose medical records did not contain a biological test result, i.e. unknown HIV and/or unknown HCV status, the following information was available: sex, age, continent of birth and prison establishment type (short, medium or long term sentences, female-only or male-only). Univariate and multivariate analyses (using logistic regression) were performed to compare sex and age of persons whose medical records contained a biological test result (for HIV or HCV) with those whose records did not contain such a result.

All analyses were performed using STATA 11 software.

Results

On the sampling day (15 May 2010), the total number of French prison establishments (for prisoners with short, medium and long sentences) equalled 188 and held 60,975 people. Of these, 27 prisons were randomly selected with specific oversampling conditions, from which 2,154 prison inmates were selected.

Characteristics of the prison population in France on the sampling day

The majority of prisoners were men (97%), and the average age of prison inmates was 34 years (interquartile range: 25–42 years; standard deviation: 0.56). People born in France accounted for 76% of inmates,

Table

Estimated number of prison inmates infected with human immunodeficiency virus or hepatitis C virus, and prevalence by sex, age group, and continent of birth, France, May 2010 (n=1,876)

	Study participants n[a]	HIV prevalence % (95% CI)	HIV-infected inmates n[b]	HCV prevalence % (95% CI)	HCV-infected inmates n[b]
Total	**1,876**	**2.0 (1.0–4.2)**	**1,234**	**4.8 (3.5–6.5)**	**2,927**
Sex[c]					
Males	1,607	2.0 (0.9–4.3)	1,173	4.5 (3.3–6.3)	2,658
Females	267	2.6 (0.7–8.8)	61	11.8 (8.5–16.1)	239
Age group[d]					
18–21	162	0	0	0	0
22–25	328	1.1 (0.1–7.7)	110	1.0 (0.2–4.5)	97
26–30	369	1.7 (0.3–8.3)	216	2.3 (1.3–4.1)	290
31–40	471	3.2 (1.1–8.6)	538	6.8 (3.9–11.3)	1,137
41–50	332	3.7 (1.3–10.3)	357	11.6 (7.2–18.1)	1,093
≥50	203	0.2 (0.0–1.66)	13	4.5 (1.5–12.3)	310
Continent of birth[d]					
France	1,388	1.1 (0.4–2.5)	487	5.0 (3.7–6.7)	2,306
Sub-Saharan Africa	90	15.4 (6.6–31.8)	522	0	0
North Africa	149	3.2 (0.4–24.6)	166	5.9 (2.3–14.3)	294
Americas	94	3.5 (0.8–13.5)	58	0	0
Asia	31	0	0	12.4 (2.1–48.2)	139
Eastern Europe	59	0	0	12.3 (4.2–30.9)	188
Western Europe	47	0.04 (0.005–0.4)	1	0	0

CI: confidence interval; HCV: hepatitis C virus; HIV: human immunodeficiency virus.

[a] Number of observations in the sample.

[b] Extrapolated number of inmates, taking into account the weight sampling design and an adjustment by post stratification on the total prison population.

[c] Sex unknown for two inmates.

[d] Age group and continent of birth were unknown for 11 and 18 cases, respectively.

while those born in north Africa and sub-Saharan Africa accounted for 9% and 5%, respectively. For people born in eastern Europe and western Europe the percentages were 3.4% and 2.7%, respectively.

Study population

Of the 2,154 prisoners included, 57 refused to take part in the survey. Questionnaires completed from medical records numbered 1,876 (87%), one questionnaire per prisoner, and represented 1,607 men and 267 women; sex was unknown for two inmates. Non-completed questionnaires included 221 cases for whom the medical records could no longer be consulted at the time of the survey, because the prisoner's conditions of imprisonment had changed in the period since sampling.

Of the 1,876 questionnaires used, information on HIV and HCV infection status was missing from 28% and 30% of records, respectively.

Comparison of persons with and without biological test result in their medical records

In the univariate and multivariate analysis, prisoners whose medical records did not contain any information on HIV or HCV status were not significantly different from those whose medical records did, in terms of sex, age, and continent of birth (data not shown). However, HIV or HCV test results were two to four times as often absent from medical records of prisoners held in establishments for short and medium length sentences than of those held in prisons for long sentence prisoners.

HIV prevalence and characteristics of HIV-infected persons

HIV prevalence

HIV prevalence was estimated at 2.0% (95% confidence interval (CI): 1.0–4.2) (Table). Prevalence was higher in women than for men, 2.6% (95% CI: 0.7–8.8) and 2.0% (95% CI: 0.9–4.3), respectively, but the difference was not significant. HIV prevalence increased with age up to 50 years from 0% among the 18–21 year-olds to 3.7% among the 41–50 year-olds. HIV prevalence varied according to continent of birth, being highest among individuals born in sub-Saharan Africa (15.4%). Among those born in France, HIV prevalence was 1.1% and was not significantly different from that estimated for people born in North Africa (3.2%) and those born in the Americas (3.5%). No inmate born in Asia or eastern Europe was infected with HIV in our study.

Characteristics and treatment of HIV-infected prisoners

Twenty-six individuals were identified as HIV-infected, with a mean age of 36 years (95% CI: 30–41.8). HIV-infected women were significantly older than men (44 years; 95% CI: 43–46) versus 35 years (95% CI: 29–42). For a large proportion 66.4% (95% CI: 46.1–82.0) the mode of transmission was unknown. When this information was available, heterosexual intercourse was the transmission mode in the majority of cases (74.7%; 95% CI: 15.5–98.0).

The mean time since diagnosis of seropositivity was nine years (range: 4.7–13.5 years), and 24% of HIV-infected prisoners had been diagnosed during imprisonment. Nearly one third (28.4%, 95% CI: 9.3–60.4) of inmates had been diagnosed with acquired immunodeficiency syndrome (AIDS), 55.3% (95% CI: 26.5–80.9) were asymptomatic and 16.4% (95% CI: 2.0–65.0) were symptomatic (non-AIDS). Three quarters of all HIV-infected had CD4 lymphocyte counts below 350/mm^3 (74%; 95% CI: 44.3–90.8), 8% (95% CI: 1.7–3.4) between 350/mm^3 and 500/mm^3, and 18.2% (95% CI: 5.5–45.8) had CD4 above 500/mm^3.

A majority of HIV-positive inmates (75%) were receiving antiretroviral treatment for HIV at the time of the survey. Among inmates with a CD4 count below 350/mm^3, the proportion of people receiving antiretroviral treatment was 72%.

HCV prevalence and characteristics of HCV-infected persons

HCV prevalence

HCV prevalence was estimated at 4.8% (95% CI: 3.5–6.5) and increased significantly with age up to 50 years, from 0% in the age group 18–21 years to 11.6% in the age group 41–50 years. Prevalence was significantly higher among women than among men, 11.8% (95% CI: 8.5–16.1) and 4.5% (95% CI: 3.3–6.3), respectively (Table). Prevalence varied by continent of birth, being highest among individuals born in Asia (12.4%) and in eastern Europe (12.3%). Prevalence among people born in France versus north Africa was not significantly different, 5.0% and 5.9%, respectively. Inmates born in sub-Saharan Africa and the Americas were not infected with HCV in our study.

Characteristics and treatment of HCV-infected prisoners

In all, 63 people were identified as HCV-infected. Their mean age was 40.7 years (95% CI: 37–44), which was older than the HCV-seronegative individuals (34.3 years). HCV seropositive women were, younger than males (38 versus 41 years). The main mode of transmission was drug use (70.2%; 95% CI: 48.9–85.3). In HCV-infected prisoners, 8.2% (95% CI: 1.6–32.7) of transmission was due to blood transfusion and tattooing. The transmission mode was unknown for 22.0% (95% CI: 11.5–37.0) of the cases.

The mean time since HCV diagnosis was 6.8 years (3.8–9.8 years), and 21.2% (95% CI: 9.4–41.2)of HCV-infected prisoners had been diagnosed during imprisonment. Overall, 44% (95% CI: 23.3–68.2) of prisoners with HCV had received, or were receiving, treatment.

HCV RNA quantification was reported in the medical records of the majority of HCV prisoners (information missing from 6% of records), and was positive for almost half of them (46%; 95% CI: 27.3–66.5). Nearly 2.5% of prison inmates had viraemic HCV.

Among prisoners with chronic hepatitis, i.e. with a persistent positive HCV RNA quantification, approximately half (41%) [18–68] had undergone an evaluation for fibrosis by invasive or non-invasive methods in the previous 12 months and 36% were currently receiving treatment.

The prevalence of HIV- HCV co-infection among inmates was low (0.08%; 95% CI: 0.00–0.65).

Discussion

This study is the first to estimate HIV and HCV prevalence among all prison inmates in France and to describe the characteristics of those infected. National HIV prevalence, estimated at 2%, corresponded to 1,233 HIV-infected persons in the total prison population of 60,975 in mainland France and overseas. HCV prevalence, estimated at 4.8%, corresponded to 2,927 HCV-infected persons, in most cases contaminated through drug use.

Our study of prison establishments and prison inmates based on a two-stage sampling design and post-stratification adjustment, enabled us to produce HIV and HCV estimates for the entire prison population in French and French overseas prisons i.e. including those not included in the sample. Prevalence estimates were produced for a number of categories, notably sex, age, and continent of birth. Indeed, this methodology could be used to estimate national prevalence in other countries, subject to the availability of a sampling frame comprising an exhaustive list of prison establishments and a list of prison inmates.

A number of limitations of this study need to be noted. Firstly, no biological tests were performed in our study because performing biological tests for research purposes in France requires informed written consent of participants. Currently, ethical committees in France consider that a request for ‘informed’ consent is incompatible with the status of being a prisoner, i.e. persons who are deprived of their liberty. This position could change in the future. Therefore, the HIV and HCV infection status was obtained from medical records. Although HIV and HCV testing is routinely offered to all prisoners in France, information about the status of these infections was missing from 30% of medical records. The absence of a serological test result from the medical records does not necessarily mean that

a test was not performed or offered; a test may have been performed but the result not recorded, or it may have been offered but refused by the prisoner, or performed as part of an anonymous and free medical visit. However, although the proportion not supplying this information was non-negligible, this does not necessarily mean our estimates were biased, since multivariate analysis of inmates without a serological result showed no significant difference in terms of sex, age or continent of birth, variables usually associated with HIV and HCV infection in the literature. Test results were more often lacking in medical records in establishments for short-term and medium-term sentences. This may have occurred because shorter incarceration periods limit the available time for testing.

Secondly, a high proportion of medical records (66%) for HIV infected persons had no information about transmission mode. This is perhaps due to physicians' awareness of the sensitive nature of recording such data in prisons, e.g. homosexuality is still a taboo subject in prisons. Finally, another limitation of the study is the lack of collected variables associated with HIV and/or HCV transmission such as high-risk behaviours, tattooing etc. and the lack of information about the frequency of imprisonment.

HIV and HCV prevalence among prisoners in France is approximately six times higher than in the general population, an observation consistent with those for other countries [3,5,9]. In 2009, HIV prevalence in the general French population was estimated at 0.35% [23], while in 2004, the prevalence of anti-HCV antibodies in the population was estimated at 0.8% (95% CI: 0.6–1.1) [24].

HIV prevalence in prisoners of high-income countries range from 0% in prisons in Denmark and Northern Ireland [13,25] to 8–10% in Italy and Portugal [26,27]. HIV prevalence among French prison inmates is close to that observed for prisons in North America, ranging from 1% to 2.5% according to the year of the study [1,4,5,8,11,28,29] and higher than the 0.4% reported in the United Kingdom [30,31] and in Australia [32,33]. To date, French studies in the published international literature have been based on multi-round surveys conducted in a single prison establishment in Marseille in south-eastern France, with prevalences of 4%–11% depending on the year [9,15,16] and a single-round survey in a single prison establishment in Caen, in northwestern France (prevalence 0%) [19]. Therefore, it is not possible to compare results from these surveys, conducted in a single establishment, with our HIV prevalence estimate conducted at a national level.

HCV prevalence in prison inmates of high-income countries also varies widely; an explanation may be differences in the proportions of inmates who use drugs. HCV estimates range from 1% to 50% [3,4,7,8,11-14,25-27,30,32,34]. In France, three studies [19,20,35] found prevalence rates between 4% and 6.9% in 2003, which are close to that observed in our study.

There is strong consensus in the literature that HIV and HCV prevalence is consistently higher among female prisoners, probably reflecting drug use [3,5,7,11,36]. Among the female prisoners in the Prévacar survey, almost one in 10 was infected with HCV. In addition, our study found that HIV and HCV prevalence increased with age, which is also consistent with published literature. Prevalence rates also varied depending on the prisoners' continent of birth, although the small numbers mean that the rates associated with continent of birth were subject to greater inaccuracy (this is reflected by large confidence intervals). HIV prevalence among prisoners born in sub-Saharan Africa was particularly high, over 15%, partly owing to the generalised HIV epidemic in that region. Similarly, HCV prevalence was high, over 12% among prisoners born in Asia, a region with high HCV endemicity, and in eastern Europe, where there is a large-scale epidemic among drug users.

The majority of HIV-infected prisoners in this study had been diagnosed seropositive several years earlier and were at an advanced stage of immunodeficiency: 75% had a CD4 count below 350/mm^3 when the study took place. Among those 75% inmates, three quarters were receiving antiretroviral treatment. Similarly, HCV-positive prisoners had been diagnosed with hepatitis several years before the study took place (on average seven years). Nearly half of them had developed chronic hepatitis. Overall, half of the HCV-infected prisoners had received or were receiving treatment for hepatitis C. These results suggest that treatment for HCV and HIV infections are available for inmates in French prisons, but could be reinforced among inmates with CD4 counts below 350/mm^3 and among those with active HCV infection.

The method developed for this survey enabled us to estimate HIV and HCV prevalences in French prisons at a national level. It could be used for other infectious diseases, in particular tuberculosis and hepatitis B. Nearly 2.5% of prison inmates in France have viraemic HCV, and the risk of HCV transmission to other prisoners is exacerbated by widespread risk behaviours such as tattooing and the sharing of shaving equipment [3,37]. It is important that prison establishments maximise efforts to limit the spread of HCV by introducing prevention and harm reduction measures [38,39]. The moment of incarceration provides an ideal opportunity for testing and treating HIV and HCV in order to limit the spread of HCV and to improve the prognosis of infected patients.

Members of the Prévacar Group:

J Bouscaillou, G Braz, M Clément, E Chaigne, JC Cognet, D de Galard, S Essid, B Faliu, C Gasiglia, L Lavin, D Legrand, E Lucas, C Michon, F Moreau, H Morfini, R Nouiouat, F Pilorgé, P Pouyanne, C Vuldy.

Acknowledgements

We would like to thank the French national prison service for its support (Dominique de Galard, Jean-Claude Cognet, Dimitri Legrand). We would also like to thank the staff of all the participating prison health facilities, Martine Clément, the physicians who conducted the survey in the prisons (Bernard Faliu, Lionel Lavin, Christophe Michon from the Ministry of health), and the members of the survey steering committee (Gregory Braz, Emeline Chaigne, Sandra Essid, Caroline Gasiglia, Hélène Morfini, François Moreau, Rhida Nouiouat, Fabrice Pilorgé, Pierre Pouyanne, Chantal Vuldy). We also thank Charlotte Verdot. Finally, our thanks to Jude Sweeney for the English revision and editing of the manuscript.

Funding

This work was supported by the French Ministry of Health and the Institut de Veille Sanitaire (InVS). The funding for the study came jointly from the Ministry of Health and the InVS. The survey steering committee included representatives of the prison physicians' professional organisations, prison authorities, infectious disease specialists, and representatives of treatment providers and civil society (anti-AIDS agencies).

References

1. Fazel S, Baillargeon J. The health of prisoners. Lancet. 2011;377(9769):956-65. http://dx.doi.org/10.1016/S0140-6736(10)61053-7.
2. Dolan K, Kite B, Black E, Aceijas C, Stimson GV. HIV in prison in low-income and middle-income countries. Lancet Infect Dis. 2007;7(1):32-41. http://dx.doi.org/10.1016/S1473-3099(06)70685-5.
3. Vescio MF, Longo B, Babudieri S, Starnini G, Carbonara S, Rezza G, et al. Correlates of hepatitis C virus seropositivity in prison inmates: a meta-analysis. J Epidemiol Community Health. 2008;62(4):305-13. http://dx.doi.org/10.1136/jech.2006.051599. PMid:18339822.
4. Gough E, Kempf MC, Graham L, Manzanero M, Hook EW, Bartolucci A, et al. HIV and hepatitis B and C incidence rates in US correctional populations and high risk groups: a systematic review and meta-analysis. BMC Public Health. 2010;10:777. http://dx.doi.org/10.1186/1471-2458-10-777. PMid:21176146. PMCid:PMC3016391.
5. Weinbaum CM, Sabin KM, Santibanez SS. Hepatitis B, hepatitis C, and HIV in correctional populations: a review of epidemiology and prevention. AIDS. 2005;19 Suppl 3:S41-6. http://dx.doi.org/10.1097/01.aids.0000192069.95819.aa. PMid:16251827.
6. Direction de l'administration pénitentiaire. [Prison Directorate]. Les chiffres clés de l'administration pénitentiaire au 1er janvier 2009. [Key figures of the prison administration on 1 January 2009]. Paris: Ministère de la Justice; 2009. 15 p. French. Available from: http://www.justice.gouv.fr/art_pix/Chiffresclesjanv2009.pdf
7. Baillargeon J, Wu H, Kelley MJ, Grady J, Linthicum L, Dunn K. Hepatitis C seroprevalence among newly incarcerated inmates in the Texas correctional system. Public Health. 2003;117(1):43-8. http://dx.doi.org/10.1016/S0033-3506(02)00009-4
8. Macalino GE, Vlahov D, Sanford-Colby S, Patel S, Sabin K, Salas C, et al. Prevalence and incidence of HIV, hepatitis B virus, and hepatitis C virus infections among males in Rhode Island prisons. Am J Public Health. 2004;94(7):1218-23. http://dx.doi.org/10.2105/AJPH.94.7.1218. PMid:15226146. PMCid:PMC1448424.
9. Rotily M, Weilandt C, Bird SM, Kall K, Van Haastrecht HJ, Iandolo E, et al. Surveillance of HIV infection and related risk behaviour in European prisons. A multicentre pilot study. Eur J Public Health. 2001;11(3):243-50. http://dx.doi.org/10.1093/eurpub/11.3.243. PMid:11582600.
10. Allwright S, Bradley F, Long J, Barry J, Thornton L, Parry JV. Prevalence of antibodies to hepatitis B, hepatitis C, and HIV and risk factors in Irish prisoners: results of a national cross sectional survey. BMJ. 2000;321(7253):78-82. http://dx.doi.org/10.1136/bmj.321.7253.78. PMid:10884256. PMCid:PMC27426.
11. Calzavara L, Ramuscak N, Burchell AN, Swantee C, Myers T, Ford P, et al. Prevalence of HIV and hepatitis C virus infections among inmates of Ontario remand facilities. CMAJ. 2007;177(3):257-61. http://dx.doi.org/10.1503/cmaj.060416. PMid:17664449. PMCid:PMC1930192.
12. Poulin C, Alary M, Lambert G, Godin G, Landry S, Gagnon H, et al. Prevalence of HIV and hepatitis C virus infections among inmates of Quebec provincial prisons. CMAJ. 2007;177(3):252-56. http://dx.doi.org/10.1503/cmaj.060760. PMid:17664448. PMCid:PMC1930200.
13. Danis K, Doherty L, McCartney M, McCarrol J, Kennedy H. Hepatitis and HIV in Northern Ireland prisons: a cross-sectional study. Euro Surveill 2007;12(1):pii=674. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=674
14. Pallás J, Fari-as-Alvarez C, Prieto D, Llorca J, Delgado-Rodríguez M. Risk factors for monoinfections and coinfections with HIV, hepatitis B and hepatitis C viruses in northern Spanish prisoners. Epidemiol Infect. 1999;123(1):95-102. http://dx.doi.org/10.1017/S0950268899002538. PMid:10487645. PMCid:PMC2810732.
15. Rotily M, Galinier-Pujol A, Obadia Y, Moatti JP, Toubiana P, Vernay-Vaisse C, et al. HIV testing, HIV infection and associated risk factors among inmates in south-eastern French prisons. AIDS. 1994;8(9):1341-4. http://dx.doi.org/10.1097/00002030-199409000-00020. PMid:7802991.
16. Rotily M, Vernay-Vaisse C, Bourliere M, Galinier-Pujol A, Rousseau S, Obadia Y. HBV and HIV screening, and hepatitis B immunization programme in the prison of Marseille, France. Int J STD AIDS. 1997;8(12):753-9. http://dx.doi.org/10.1258/0956462971919228. PMid:9433949.
17. Hedouin V, Gosset D. Infection par le virus de l'hépatite C en milieu carcéral. [Infection with hepatitis C virus in a prison environment. A prospective study in Loos-lez-Lille, France]. Gastroenterol Clin Biol. 1998;22(1):55-8. French. PMid:9762167.
18. Claudon-Charpentier A, Hoibian M, Glasser P, Lalanne H, Pasquali JL. La population toxicomane incarcérée: séroprévalences du virus d'immunodéficience humaine et des virus des hépatites B et C peu après la mise sur le marché de la buprénorphine. [Drug-addicted prisoners: seroprevalence of human immunodeficiency virus and hepatitis B and C virus soon after the marketing of buprenorphine]. Rev Med Interne. 2000;21(6):505-9. French. http://dx.doi.org/10.1016/S0248-8663(00)89225-5
19. Verneuil L, Vidal JS, Ze BR, Vabret A, Petitjean J, Leclercq R, et al. Prevalence and risk factors of the whole spectrum of sexually transmitted diseases in male incoming prisoners in France. Eur J Clin Microbiol Infect Dis. 2009;28(4):409-13. http://dx.doi.org/10.1007/s10096-008-0642-z. PMid:18998176.
20. Remy AJ. Amélioration du dépistage et du traitement de l'hépatite C en prison. Enquête comparative entre 2000 et 2003. [Hepatitis C in prison settings: screening and therapy are improving. Comparative survey between 2000 and 2003]. Presse Med. 35(9 Pt 1) :1249-54. French.
21. Rich JD, Wohl DA, Beckwith CG, Spaulding AC, Lepp NE, Baillargeon J, et al. HIV-related research in correctional populations: now is the time. Curr HIV/AIDS Rep. 2011;8(4):288-96. http://dx.doi.org/10.1007/s11904-011-0095-3. PMid:21904902. PMCid:PMC3208731.
22. Tillé Y. Sampling algorithms.Springer Series in Statistics. New York: Springer, 2006. PMCid:PMC2360768.
23. Yeni P (editor). Rapport 2010. Prise en charge médicale des personnes infectées par la VIH: Recommandations du groupe d'experts. [2010 report. Medical care of persons infected with HIV: recommendations of the expert group]. Paris: La documentation Française; 2010. p. 24-34. French. Available from: http://www.sante.gouv.fr/IMG/pdf/Rapport_2010_sur_la_prise_en_charge_medicale_des_personnes_infectees_par_le_VIH_sous_la_direction_du_Pr-_Patrick_Yeni.pdf
24. Meffre C, Le Strat Y, Delarocque-Astagneau E, Dubois F, Antona D, Lemasson JM, et al. Prevalence of hepatitis B and hepatitis C virus infections in France in 2004: social factors are important predictors after adjusting for known risk factors. J Med Virol. 2010;82(4):546-55. http://dx.doi.org/10.1002/jmv.21734. PMid:20166185.
25. Christensen PB, Krarup HB, Niesters HG, Norder H, Georgsen J. Prevalence and incidence of bloodborne viral infections among Danish prisoners. Eur J Epidemiol. 2000;16(11):1043-9. http://dx.doi.org/10.1023/A:1010833917242. PMid:11421474.

26. Babudieri S, Longo B, Sarmati L, Starnini G, Dori L, Suligoi B, et al. Correlates of HIV, HBV, and HCV infections in a prison inmate population: results from a multicentre study in Italy. J Med Virol. 2005;76(3):311-7. http://dx.doi.org/10.1002/jmv.20375. PMid:15902712.
27. Barros H, Ramos E, Lucas R. A survey of HIV and HCV among female prison inmates in Portugal. Cent Eur J Public Health. 2008;16(3):116-20. PMid:18935775.
28. Wilper AP, Woolhandler S, Boyd JW, Lasser KE, McCormick D, Bor DH, et al. The health and health care of US prisoners: results of a nationwide survey. Am J Public Health. 2009;99(4):666-72. http://dx.doi.org/10.2105/AJPH.2008.144279. PMid:19150898. PMCid:PMC2661478.
29. VanHandel M, Beltrami JF, MacGowan RJ, Borkowf CB, Margolis AD. Newly identified HIV infections in correctional facilities, United States, 2007. Am J Public Health. 2012;102 Suppl 2:S201-4. http://dx.doi.org/10.2105/AJPH.2011.300614. PMid:22401522.
30. Weild AR, Gill ON, Bennett D, Livingstone SJ, Parry JV, Curran L. Prevalence of HIV, hepatitis B, and hepatitis C antibodies in prisoners in England and Wales: a national survey. Commun Dis Public Health. 2000;3(2):121-6. PMid:10902255.
31. Edwards A, Curtis S, Sherrard J. Survey of risk behaviour and HIV prevalence in an English prison. Int J STD AIDS. 1999;10(7):464-6. http://dx.doi.org/10.1258/0956462991914474. PMid:10454182.
32. Butler TG, Dolan KA, Ferson MJ, McGuinness LM, Brown PR, Robertson PW. Hepatitis B and C in New South Wales prisons: prevalence and risk factors. Med J Aust. 1997;166(3):127-30. PMid:9059433.
33. Crofts N, Stewart T, Hearne P, Ping XY, Breshkin AM, Locarnini SA. Spread of bloodborne viruses among Australian prison entrants. BMJ. 1995;310(6975):285-8. http://dx.doi.org/10.1136/bmj.310.6975.285. PMid:7866168. PMCid:PMC2548691.
34. Saiz de la HP, Marco A, Garcia-Guerrero J, Rivera A. Hepatitis C and B prevalence in Spanish prisons. Eur J Clin Microbiol Infect Dis. 2011;30(7):857-62. http://dx.doi.org/10.1007/s10096-011-1166-5. PMid:21274586.
35. Direction de l'Hospitalisation et de l'Organisation des Soins (DHOS). [Directorate of Hospitalisation and Care Organization]. Enquête un jour donné sur les personnes détenues atteintes par le VIH et le VHC en milieu pénitentiaire: résultats de l'enquête de juin 2003. [Survey on a given day on prisoners infected with HIV and HCV in prisons: results of the survey in June 2003]. Paris: DHOS; 2004. French. Available from: http://www.sante.gouv.fr/enquete-un-jour-donne-sur-les-personnes-detenues-atteintes-par-le-vih-et-le-vhc-en-milieu-penitentiaire.html
36. Wu ZH, Baillargeon J, Grady JJ, Black SA, Dunn K. HIV Seroprevalence among newly incarcerated inmates in the Texas correctional system. Ann Epidemiol. 2001;11(5):342-6. http://dx.doi.org/10.1016/S1047-2797(01)00210-1.
37. Teutsch S, Luciani F, Scheuer N, McCredie L, Hosseiny P, Rawlinson W, et al. Incidence of primary hepatitis C infection and risk factors for transmission in an Australian prisoner cohort. BMC Public Health. 2010;10:633. http://dx.doi.org/10.1186/1471-2458-10-633. PMid:20964864. PMCid:PMC2975656.
38. Michel L, Carrieri MP, Wodak A. Harm reduction and equity of access to care for French prisoners: a review. Harm Reduct J. 2008;5:17. http://dx.doi.org/10.1186/1477-7517-5-17. PMid:18495018. PMCid:PMC2430551.
39. Michel L, Jauffret-Roustide M, Blanche J, Maguet O, Calderon C, Cohen J, et al. Limited access to HIV prevention in French prisons (ANRS PRI2DE): implications for public health and drug policy. BMC Public Health. 2011;11:400. http://dx.doi.org/10.1186/1471-2458-11-400. PMid:21619573. PMCid:PMC3128573.

Prevalence of *Coxiella burnetii* in women exposed to livestock animals, Denmark, 1996 to 2002

S Yde Nielsen (stineyde@dadlnet.dk)[1,2], K Mølbak[3], A M Nybo Andersen[4], T Brink Henriksen[5], B Kantsø[6], K A Krogfelt[6], N H Hjøllund[1,7]

1. Department of Occupational Medicine, Regional Hospital West Jutland, Herning, Denmark
2. Perinatal Epidemiology Research Unit, Aarhus University Hospital, Skejby, Aarhus, Denmark
3. Department of Infectious Epidemiology, Statens Serum Institut, Copenhagen, Denmark
4. Section of Social Medicine, Department of Public Health, University of Copenhagen, Copenhagen, Denmark
5. Perinatal Epidemiology Research Unit and Department of Pediatrics, Aarhus University Hospital, Skejby, Aarhus, Denmark
6. Department of Microbiological Surveillance and Research, Statens Serum Institut, Copenhagen, Denmark
7. Department of Clinical Epidemiology, Aarhus University Hospital, Aarhus, Denmark

Citation style for this article:
Yde Nielsen S, Mølbak K, Nybo Andersen AM, Brink Henriksen T, Kantsø B, Krogfelt KA, Hjøllund NH. Prevalence of Coxiella burnetii in women exposed to livestock animals, Denmark, 1996 to 2002. Euro Surveill. 2013;18(28):pii=20528. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20528

Article submitted on 20 September 2012 / published on 11 July 2013

Q fever is a zoonotic infection which can pose a danger to pregnant women. To our knowledge, Denmark has never experienced a clinically verified Q fever outbreak. We aimed to quantify risk of infection in pregnant women occupationally and environmentally exposed to *Coxiella burnetii*. The Danish National Birth Cohort collected blood samples from 100,418 pregnant women in the period 1996 to 2002. We sampled 195 women with occupational exposure to livestock (veterinarians and female farmers), 202 women with domestic exposure (dairy cattle and/or sheep) and a random sample of 459 unexposed women. Samples were screened for antibodies against *C. burnetii* by commercial enzyme-linked immunosorbent assay. Positive samples were confirmed by immunofluorescence (cut-off titre ≥1:128). The proportion of seropositive women was higher in the occupationally exposed (47.2% seropositive; relative risk (RR): 9.8; 95% confidence interval (CI): 6.4–15.2) and the domestically exposed population (32.2% seropositive; RR: 6.7; 95% CI: 4.3–10.6) than in unexposed women (4.8% seropositive). We found a high prevalence of antibodies to *C. burnetii* among pregnant women with occupational or domestic exposure to cattle and/or sheep compared with unexposed pregnant women. Our findings suggest that contact to livestock is a risk factor for *C. burnetii* infection in Denmark.

Introduction

Most emerging infectious diseases are of zoonotic origin [1], and populations at particularly high risk often include individuals with occupational exposure to live animals, such as veterinarians, farmers and those living in close contact with domestic livestock. Q fever, caused by *Coxiella burnetii*, is a disease of particular concern for pregnant women because infection in pregnancy is suspected to be a potential cause of foetal morbidity and mortality. French case studies have suggested risk of miscarriage, intrauterine growth retardation, oligohydramnion, stillbirth and premature delivery in untreated pregnancies [2-4]. Recent studies have not found any association between presence of antibodies against *C. burnetii* and adverse pregnancy outcome, but knowledge on the topic is sparse [5-9]. For healthy humans, Q fever infection often has a mild, influenza-like course, but pneumonia is also common. Immunocompromised patients and patients with pre-existing valvulopathy or vascular defects are at risk of a more severe course of the infection [10,11].

In small ruminants, infection with *C. burnetii* is known to cause miscarriage, retained placenta, endometritis and infertility, and placentas of infected animals contain high numbers of bacteria [12,13]. Human infection is usually acquired through inhalation of contaminated aerosols from infected animals, which contaminate the environment through excretion of bacteria in large amounts in byproducts during birth, especially placenta [10,11,14]. The risk of infection with *C. burnetii* has been related to particular occupations with close contact to the organism's primary reservoirs, such as domesticated livestock animals. Examples include veterinary practice and farming [15,16].

Q fever is most likely endemic worldwide, but unbiased estimates from relevant populations are scarce because most reports on incidence and prevalence are reported from regions with outbreaks or with particular medical or scientific interest in the infection [2]. In Denmark, Q fever has previously been considered a rare and imported disease, but testing for antibodies in livestock animals since 2003 has indicated that the infection is widespread. A recent study found a prevalence of 59% antibody-positive animals from 100 randomly selected dairy herds [17].

When conducting a risk assessment, it is important to quantify the risk of infection in exposed populations.

The aim of the present study was to investigate the prevalence of elevated antibody titres against *C. burnetii* in Denmark in occupationally and domestically exposed women compared with unexposed women sampled from a population based study of pregnant women.

Methods

Study participants

The Danish National Birth Cohort (DNBC), a nationwide cohort of 100,418 pregnant women and their offspring [18], served as base for sampling of the study population. Enrolment in the DNBC took place between 1996 and 2002. All Danish pregnant women were invited for the study in connection with the first antenatal visit to the general practitioner. Information on exposures before and during the early part of pregnancy was collected by means of a computer-assisted telephone interview scheduled to take place in gestational week 12. Interviews included data on reproductive history, age, smoking status, domestic contact to animals and very detailed questions regarding occupational exposure to different animals (interview forms are available at the DNBC website).

Women who confirmed having worked on a farm with live animals during their pregnancy or up to three months before becoming pregnant, were further questioned about the type of animals, the size of the herd, occupation, etc. During pregnancy, two blood samples were collected, one around gestational weeks 6 to 12, the second around gestational week 24; samples were stored in a biobank. A detailed description of the cohort can be found elsewhere [18].

We sampled three groups from the DNBC cohort (Figure 1):

- Women with self-reported occupational exposure to livestock (n=195), i.e. veterinarians (n=118) and women who worked on a farm with at least 40 dairy cattle (n=77);
- Women with self-reported domestic exposure to livestock (n=202), i.e. cattle (n=180), sheep (n=22) or both (n=13), who were living on a farm and cohabiting with a farmer, but did not have occupational exposure to these animals;
- A randomly sampled reference group of women (n=461). Two of these were domestically exposed to animals and were consequently reclassified as such, leaving 459 controls.

It was a prerequisite for all three groups that the women had participated in the interview in early pregnancy and had delivered a blood sample to the biobank.

In order to evaluate a possible association between geographic area and seropositivity, the participants were classified using the nomenclature of territorial units for statistics (NUTS3) [19], which divides the regions of Denmark into 11 areas. These were used in a definition of urban versus rural residence.

Detection of antibodies against *C. burnetii*

The diagnosis of Q fever relies upon serology. *C. burnetii* expresses two groups of antigens, phase I and phase II. In acute Q fever, antibodies against phase II antigens are initially elevated, and their titre is higher than that of antibodies against phase I antigens. As with most other infections, IgM antibodies appear first. In chronically infected individuals, especially antibodies against phase I are elevated. When infected, phase II IgG and IgM antibodies are always elevated, and IgG remain positive for many years. A large study from Australia and England concluded that phase II IgG antibodies persisted after five and 12 years, respectively [20].

To determine antibodies against *C. burnetii*, we used a two-step approach. Initially, all samples were screened in a commercial enzyme-linked immunosorbent assay (ELISA). The commercial ELISA kit (Panbio, Australia, *Coxiella burnetii* (Q Fever) IgG and *Coxiella burnetii* (Q Fever) IgM) were used according to the manufacturer's instructions with minor modifications. Due to small sample size the initial total volume was smaller but same dilution factors were used.

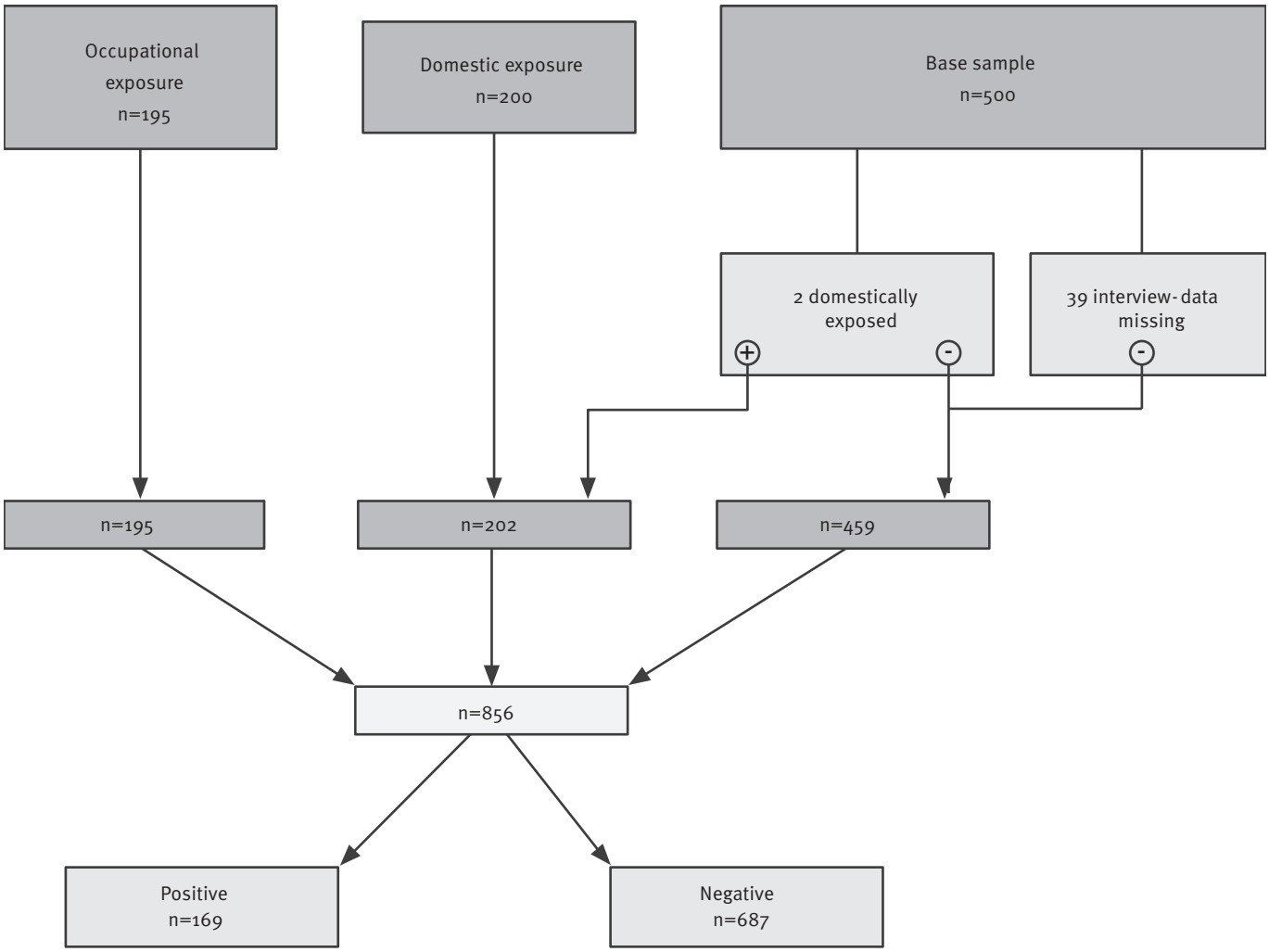
Samples which were positive for either IgG or IgM antibodies in the ELISA were confirmed with an immunofluorescence antibody test (IFA) test. When investigating the association between exposure, Q fever titres and pregnancy outcome, IFA is considered to be the gold standard. The tests (Focus Diagnostics, Q Fever IFA IgG and Q Fever IFA IgM) were performed according to the instructions provided by the manufacturer, with the following minor modifications: due to small sample volume, the 1:10-diluted samples from the ELISA were reused and further diluted as described by the manufacturer. The effect of the initial dilution in the Panbio ELISA buffer was tested on patient samples before the study and did not show any influence on the results (data not shown).

The IFA cut-off suggested by the manufacturer was not used. Since the prevalence of the infection varies between geographic areas, the cut-off suggested by the manufacturer is not necessarily suited for any given area [21]. A local cut-off adjusted to the Danish population has been defined, including negative, intermediate and positive titres [22] (Table 1). The intermediate zone was defined in order to address people with an a priori elevated risk of Q fever (such as veterinarians, farmers etc.), with intermediate titres in samples from these high-risk groups considered to be probably positive. When the ELISA-positive samples in our study were reanalysed using IFA, a modified version of this Danish cut-off was used. A sample was considered IFA-positive when antibody titres against any of the phases were 1:128 or above.

All serological analyses were performed in a certified laboratory at Statens Serum Institut, Denmark. Laboratory personnel were blinded for exposure

Figure 1

Sampling of pregnant women from the Danish National Birth Cohort, Denmark, 1996–2002 (n=856)



status, and samples were always analysed in the same batch of commercial kits.

We have conducted another study assessing pregnancy outcome in women with antibodies to *C. burnetii* compared to seronegative women [9]. This and the present study in part use the same material since the blood samples from the Danish national birth cohort is a precious commodity. However, the studies are independent studies with different study designs and objectives.

Statistical analysis

The strength of the association between exposure and positive IFA serology was expressed as a risk difference as well as a relative risk for occupational and domestic exposure compared to the reference according to the prevalence of antibodies against *C. burnetii* in pregnancy.

We included all veterinarians and women who reported occupational exposure to cattle in the occupationally exposed group. Power calculations were based on the literature and the first Danish data [23] with 11% of 1,613 people tested positive. It was assumed that the prevalence among exposed women would be 10% and 2% in the background population. A sample size of 200 exposed and 200 unexposed would yield an odds ratio of 5 that could be detected by a power of 88% at a two-sided significance level of 0.05. However, as we also wanted to use the sample for another study which required approximately 500 controls, it was decided to use all available blood samples from the reference group in both studies. All analyses were carried out using STATA statistical software, version 11.

Results

Age and distribution of urban or rural residence can be seen in Table 2. Age was normally distributed in all three groups. The median age among occupationally exposed women was 31 years (interquartile range: 28–33 years), compared with 30 years (interquartile range: 27–33 years) in domestically exposed women, and 29 years (interquartile range: 26–32 years) in the unexposed.

Table 1

Cut-off values immunofluorescence antibody test as applied in Denmark

	Negative	Intermediate	Positive
IgM phase I	‹64	64	≥128
IgM phase II	‹64	64–128	≥256
IgG phase I	‹128	128–256	≥512
IgG phase II	‹128	128–512	≥1,024

Source: [22].
In the present study, a cut-off of 1:128 was used for all phases.

When looking at age and seropositivity, the smallest proportion of IFA-positive women were found in the age group younger than 25 years (13.5% seropositives); findings from other age groups, 25 to 34 years and 35 years and older, were similar to each other (22.7% and 18.1% seropositives, respectively). There was no correlation between age and seropositivity.

Figure 2 illustrates the relationship between IgG phase II-positive ELISA and IFA results. Positive IFA results were more frequent in samples with high adjusted optical density values (OD, measuring antibody concentrations) in the ELISA.

In the confirmatory IFA analysis, 92 (47.2%; 95% confidence interval (CI): 40.0–54.4) occupationally and 65 (32.2%; 95% CI: 25.8–39.0) domestically exposed women were *C. burnetii* antibody-positive in IFA, compared with three (4.8%; 95% CI: 3.0–7.1) in the unexposed group. The risk difference between the occupationally exposed and unexposed women was 42% (95% CI: 35–50), and the occupationally exposed had a 9.8 times higher risk of being seropositive than the unexposed women (relative risk (RR): 9.8; 95% CI: 6.4–15.2). The risk difference between the domestically exposed and unexposed women was 27% (95% CI: 0.2–0.3), and the domestically exposed had a 6.7 times higher risk (95% CI: 4.3–10.6) of being seropositive than the unexposed women (Table 3).

Reporting the IFA results according to the Danish cut-off with intermediate titres classified as negative (Table 1), the trend was the same. Here the proportion of seropositive women was also significantly higher in women with occupational exposure to livestock (19% seropositive; RR: 29; 95% CI: 9.1–93.0). This was also found in women with domestic exposure to livestock (11.0% seropositive; RR: 16.7; 95% CI: 5.0–55.0) when compared with unexposed women (0.7% seropositive).

Figure 3 shows the distribution of positive IgG phase II titres in the three groups and illustrates that unexposed women had mainly titres at the lower end of positivity, whereas the higher titres were primarily found in the two groups of exposed women.

Previous versus recent infection

Among the occupationally exposed women, 89 were phase II IgG-positive, 43 were phase I IgG-positive, and 41 of them were positive in both. Three women's IgM titres against phase II antigens were positive, one of them was also positive for IgG against phase II, and another in IgG against both phases. None was phase I IgM-positive. Among the domestically exposed women, 59 were phase II IgG-positive, 30 were phase I IgG-positive, and 26 of them were positive in both phases. Three were phase II IgM-positive, with one of them also being positive for IgM against phase I, and two for IgG against phase II. One was only phase I IgM-positive. Among the unexposed women, 21 were positive for IgG against phase II, six of them were also phase I IgG-positive. One was positive for IgM against phase I as well as IgG against phase II, and one was phase II IgM-positive but negative in all other phases.

Table 2

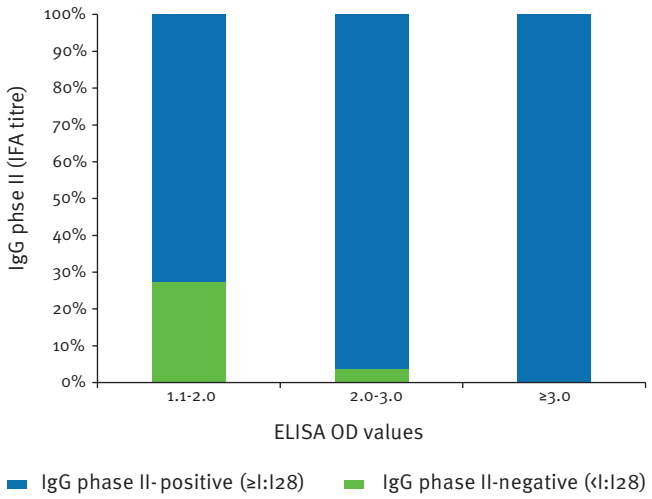
Distribution of selected characteristics among pregnant women sampled from the Danish National Birth Cohort, Denmark, 1996–2002 (n=856)

	Occupationally exposed (n=195)	Domestically exposed (n=202)	Unexposed reference (n=459)
Age (n=856)			
‹25 (n=104)	13 (6.7%)	26 (12.9%)	65 (14.2%)
25–34 (n=631)	148 (75.9%)	140 (69.3%)	343 (74.7%)
≥35 (n=121)	34 (17.4%)	36 (17.8%)	51 (11.1%)
Area of residence			
Rural (n=427)	113 (58.5%)	163 (81.9%)	151 (33.3%)
Urban (n=418)	80 (41.5%)	36 (18.1%)	302 (66.7%)

Data on area of residence not available for all participants.

Figure 2

IgG phase II antibodies against *Coxiella burnetii* in pregnant women, immunofluorescent antibody titres in relation to enzyme-linked immunosorbent assay, Denmark, 1996–2002 (n=856)



ELISA: enzyme-linked immunosorbent assay; IFA: immunofluorescence antibody test; OD: optical density.

Altogether, we mainly found serological evidence of previous infection.

Specific animal contact

Apart from working with live animals, 38 of the 118 veterinarians lived on a farm with animals; none of the veterinarians who lived on a farm had a job without animal contact.

Among the 77 female farmers who all worked on farms with at least 40 dairy cattle, 69 of them lived on cattle farms. Four of them also worked with meat cattle and five worked with sheep. All 202 women domestically exposed were living on a farm and cohabiting with a farmer; 193 of these lived on farms with cattle, 22 on farms with sheep, and 13 on farms where cattle as well as sheep were kept.

Analyses based on specific animal contact according to IFA status showed that 23 of the 31 veterinarians working with cattle were seropositive, and that the risk of being IFA positive were 2.7 times higher in veterinarians who worked with cattle compared to those who did not (RR: 2.7; 95% CI: 1.8–4.0). The positive predictive value of being seropositive being a veterinarian working with cattle was 48.9%. Among the domestically exposed women who were exposed to cattle, 64 (33.2%) were IFA-positive, and the positive predictive value of being seropositive for these women was 98.4%, whereas it was only 9.2% for domestic exposure to sheep.

Urban versus rural area

Among 427 women living in rural areas, 128 (30%) were IFA-positive compared to 48 (11.5%) seropositive among women living in urban areas. The risk of being IFA-positive was 2.6 times higher for women living in rural areas (RR: 2.6; 95% CI: 1.9–3.5). Of the unexposed women, 151 (33%) lived in rural areas. Eleven (7.3 %) of them were seropositive, compared with 11 (3.6 %) seropositive among the unexposed women living in urban areas.

Discussion

We found a high prevalence of antibodies to *C. burnetii* among pregnant women with occupational or domestic exposure to cattle or sheep compared to the prevalence in randomly selected unexposed pregnant women. The highest predictive values for being seropositive were found among pregnant veterinarians and women with domestic exposure to cattle.

In general, a higher seroprevalence has been found in studies evaluating groups handling livestock, especially veterinarians, than in studies of the background population [24-30]. In one Dutch study on veterinary students, 18.7% were seropositive [31]; in another, 65% of 189 veterinarians and veterinary students were seropositive. Greater number of hours with animal contact per week, greater number of years since

Table 3

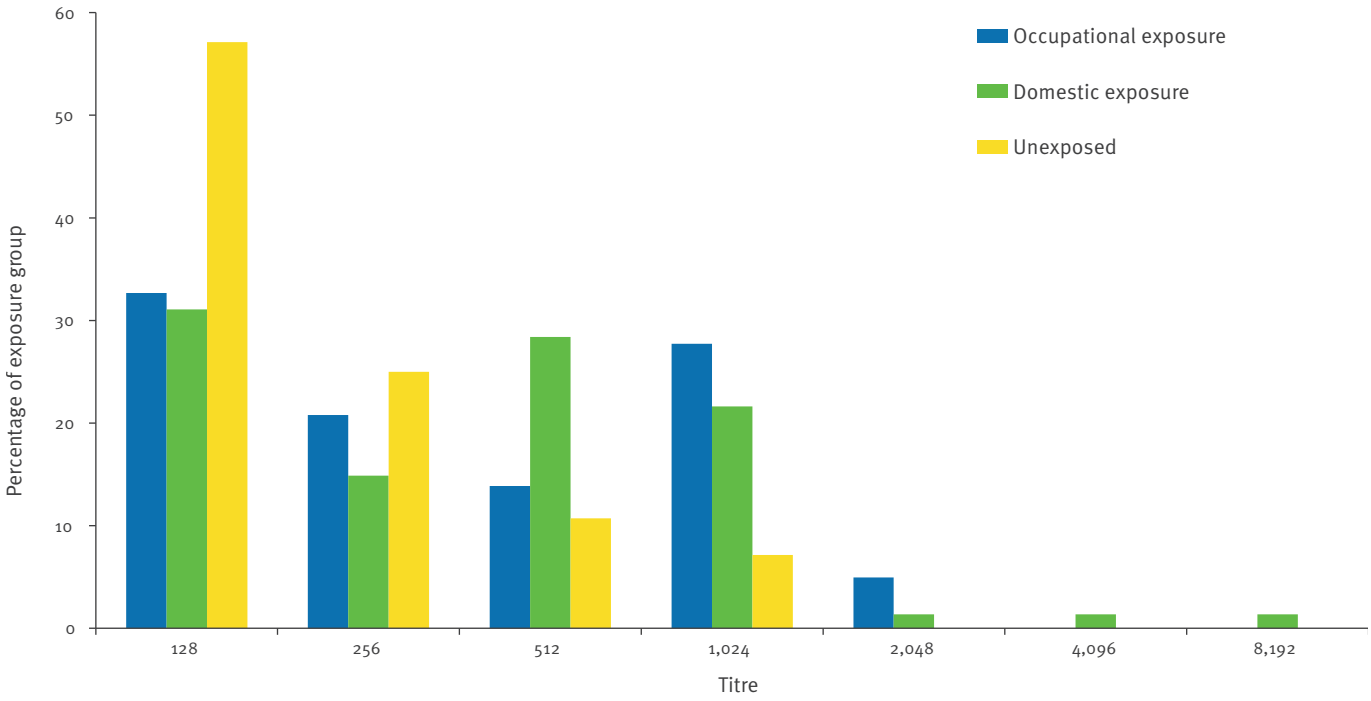
Risk difference and relative risks for pregnant women occupationally and domestically exposed to *Coxiella burnetii*, versus unexposed, Denmark, 1996–2002 (n=856)

	Occupationally exposed (n=195)	Domestically exposed (n=202)	Unexposed reference group (n=459)
IFA-negative	103 (52.8%)	137 (67.8%)	437(95.2%)
IFA-positive	92 (47.2%)	65 (32.2%)	22 (4.8%)
RD (95% CI)	0.42 (0.35–0.50)	0.27 (0.21–0.34)	Reference
RR (95% CI)	9.84 (6.37–15.20)	6.71 (4.26–10.57)	Reference

CI: confidence interval; IFA: Immunofluorescence assay; RD: risk difference; RR: relative risk.

Figure 3

Immunofluorescence IgG phase II antibody titres against *Coxiella burnetii* in pregnant women, by exposure group, Denmark, 1996–2002 (n=856)



the participants had graduated, living in a rural area, and working as practicing livestock veterinarian were risk factors in that study [32]. An American study found antibodies against *C. burnetii* in 113 (22.2%) of 508 US veterinarians. Compared with veterinarians with a small animal practice, those with a mixed practice for small and large animals and those with a practice for food animals were more likely to be seropositive. Furthermore that study found that having lived on a farm in the past, currently living on a farm, and exposure to ruminants while living on a farm were associated with seropositivity [15].

In Denmark, Q fever became a notifiable disease in animals in 2005. A change in diagnostic practices in cattle and an increasing number of cattle herds testing positive raised the level of awareness among exposed, asymptomatic humans in the period 2006–07. This increased focus on Q fever was thus due to diagnosis and testing rather than to the emergence of a new infection. In the present study, some of the blood samples analysed date back to 1996, and this indicates that *C. burnetii* is not a newly emerged pathogen in Denmark; most likely it has been common among people with contact to cattle for a long time.

The most recent blood samples from our study dated from 2002; since then, two Danish studies have examined the presence of antibodies to *C. burnetii* in humans exposed to animals. In a serological analysis of 1,613 people, tested in 2006¬–07 mainly due to relevant exposure to domestic animals, 177 (11 %) were seropositive and 180 had an equivocal result according to the Danish cut-off [33]. Another study evaluated blood samples from 2008 from people working with domestic animals and found 39 of 359 (11 %) seropositives, with the highest prevalence of antibodies (36%) among veterinarians [34]. Close contact to birth products when performing Caesarean sections and other kinds of veterinary obstetrics is a possible explanation for the higher prevalence of antibodies among veterinarians compared to domestically exposed women found in this study.

According to the authors defining the Danish cut-off [22], high risk groups, such as veterinarians and farmers, with an intermediate titre should be considered probably positive and managed as such (the predictive value of a positive result is likely to be higher in an exposed population than in the general population). Moreover, the Danish cut-off was based on the assumption that blood donors from urban areas of Denmark are not exposed to *C. burnetii*, but the prevalence of antibodies among women with no animal exposure in our study (4.8%) is rather high compared to, for instance, the seroprevalence of about 2.4% in the general population in the Netherlands before the outbreak in 2007–10 [35]. This may indicate that *C. burnetii* is generally widespread in Denmark, but could also be an argument in favour of not lowering the cut-off too much and was the rationale behind the cut-off used in this study, which was higher than in other studies [15,25,36,37] .

To our knowledge, human outbreaks of Q fever have only been described to originate from small ruminants. In France, goats and sheep have been the source of infection. The Netherlands experienced the world's largest outbreak of Q fever with more than 4,000 humans infected between 2007 and 2010 [38] and here the source of infection was goats [39].

There are different strains of *C. burnetii*, and, as for other bacteria, and some of the drivers for outbreak potential may be related to the heterogeneity in clinical outcomes, which could arise from differences in virulence and host reservoirs. The presence of strains of different pathogenicity could influence awareness of the disease and therefore partially explain the variation in illness incidence reported from different countries. In the Dutch outbreak, one genotype was suggested be responsible for the human Q fever epidemic, and this was very similar to one of the genotypes found in goats [39]. In comparison to France and the Netherlands, there are few sheep and goats in Denmark; the source of infection here is primarily cattle [40], and as far as we know, Denmark has never experienced a clinically verified Q fever outbreak.

Our study has limitations in that we did not verify positive samples with PCR or culture. But we regard the size of this cohort a major strength of this study. Also, one could argue in favour of testing random negative ELISA samples with IFA, which was not done here. However, the ELISA test was thoroughly investigated before use; the results were published by Kantsø et al [41].

In conclusion, this study found that Danish pregnant women exposed to livestock animals have significantly higher levels of antibodies against *C. burnetii* when compared to unexposed women, with the highest prevalence of antibodies found among veterinarians who worked with cattle. Our findings confirm that *C. burnetii* is not a newly emerged pathogen in Denmark and that Q fever is endemic here as probably in most other countries. Our results suggest that contact with livestock is a risk factor for *C. burnetii*. Keeping in mind the high prevalence of symptomatic human infection during the recent outbreak in the Netherlands, Q fever should be considered as a possible differential diagnosis in people with close contact to domestic animals, especially veterinarians and women domestically exposed to cattle.

References

1. Woolhouse ME, Gowtage-Sequeria S. Host range and emerging and reemerging pathogens. Emerg Infect Dis. 2005;11(12):1842-47. http://dx.doi.org/10.3201/eid1112.050997. PMid:16485468. PMCid:PMC3367654.
2. Carcopino X, Raoult D, Bretelle F, Boubli L, Stein A. Q Fever during pregnancy: a cause of poor fetal and maternal outcome. Ann N Y Acad Sci. 2009;1166:79-89. http://dx.doi.org/10.1111/j.1749-6632.2009.04519.x. PMid:19538266.
3. Carcopino X, Raoult D, Bretelle F, Boubli L, Stein A. Managing Q fever during pregnancy: the benefits of long-term cotrimoxazole therapy. Clin Infect Dis. 2007;45(5):548-55. http://dx.doi.org/10.1086/520661. PMid:17682987.
4. Angelakis E, Million M, D'Amato F, Rouli L, Richet H, Stein A, et al. Q fever and pregnancy: disease, prevention, and strain specificity. Eur J Clin Microbiol Infect Dis. 2013;32(3):361-8. http://dx.doi.org/10.1007/s10096-012-1750-3. PMid:23052984.
5. van der Hoek W, Meekelenkamp JC, Leenders AC, Wijers N, Notermans DW, Hukkelhoven CW. Antibodies against Coxiella burnetii and pregnancy outcome during the 2007-2008 Q fever outbreaks in The Netherlands. BMC Infect Dis. 2011;11:44. http://dx.doi.org/10.1186/1471-2334-11-44. PMid:21314933. PMCid:PMC3042933.
6. Munster J, Leenders A, Hamilton C, Meekelenkamp J, Schneeberger P, van der Hoek W, et al. Routine screening for Coxiella burnetii infection during pregnancy: a clustered randomised controlled trial during an outbreak, the Netherlands, 2010. Euro Surveill. 2013;18(24):pii=20504. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20504. PMid:23787163.
7. Langley JM, Marrie TJ, Leblanc JC, Almudevar A, Resch L, Raoult D. Coxiella burnetii seropositivity in parturient women is associated with adverse pregnancy outcomes. Am J Obstet Gynecol. 2003;189(1):228-32. http://dx.doi.org/10.1067/mob.2003.448. PMid:12861167.
8. Nielsen SY, Hjøllund NH, Andersen AM, Henriksen TB, Kantsø B, Krogfelt KA, et al. Presence of antibodies against Coxiella burnetii and risk of spontaneous abortion: a nested case-control study. PLoS One. 2012;7(2):e31909. http://dx.doi.org/10.1371/journal.pone.0031909. PMid:22363769. PMCid:PMC3283715.
9. Nielsen SY, Andersen AM, Molbak K, Hjollund NH, Kantso B, Krogfelt KA, et al. No excess risk of adverse pregnancy outcomes among women with serological markers of previous infection with Coxiella burnetii: evidence from the Danish national birth cohort. BMC Infect Dis. 2013;13:87. http://dx.doi.org/10.1186/1471-2334-13-87. PMid:23413787. PMCid:PMC3585700.
10. Tissot-Dupont H, Vaillant V, Rey S, Raoult D. Role of sex, age, previous valve lesion, and pregnancy in the clinical expression and outcome of Q fever after a large outbreak. Clin Infect Dis. 2007;44(2):232-37. http://dx.doi.org/10.1086/510389. PMid:17173223.
11. Fournier PE, Marrie TJ, Raoult D. Diagnosis of Q fever. J Clin Microbiol. 1998;36(7):1823-34. PMid:9650920. PMCid:PMC104936.
12. Bildfell RJ, Thomson GW, Haines DM, McEwen BJ, Smart N. Coxiella burnetii infection is associated with placentitis in cases of bovine abortion. J Vet Diagn Invest. 2000;12(5):419-25. http://dx.doi.org/10.1177/104063870001200505. PMid:11021428.
13. Berri M, Rousset E, Champion JL, Russo P, Rodolakis A. Goats may experience reproductive failures and shed Coxiella burnetii at two successive parturitions after a Q fever infection. Res Vet Sci. 2007;83(1):47-52. http://dx.doi.org/10.1016/j.rvsc.2006.11.001. PMid:17187835.
14. Parker NR, Barralet JH, Bell AM. Q fever. Lancet. 2006;367(9511):679-88. http://dx.doi.org/10.1016/S0140-6736(06)68266-4.
15. Whitney EA, Massung RF, Candee AJ, Ailes EC, Myers LM, Patterson NE, et al. Seroepidemiologic and occupational risk survey for Coxiella burnetii antibodies among US veterinarians. Clin Infect Dis. 2009;48(5):550-7. http://dx.doi.org/10.1086/596705. PMid:19191638.
16. McQuiston JH, Childs JE. Q fever in humans and animals in the United States. Vector Borne Zoonotic Dis. 2002;2(3):179-91. http://dx.doi.org/10.1089/153036602760613747. PMid:12737547.
17. Agger JF, Christoffersen AB, Rattenborg E, Nielsen J, Agerholm JS. Prevalence of Coxiella burnetii antibodies in Danish dairy herds. Acta Vet Scand. 2010;52:5. http://dx.doi.org/10.1186/1751-0147-52-5. PMid:20092653. PMCid:PMC2823749.
18. Olsen J, Melbye M, Olsen SF, Sorensen TI, Aaby P, Andersen AM, et al. The Danish National Birth Cohort--its background,

structure and aim. Scand J Public Health. 2001;29(4):300-7. http://dx.doi.org/10.1177/14034948010290040201. http://dx.doi.org/10.1080/14034940131711526 8. PMid:11775787.

19. European Commission. Eurostat. Nomenclature of territorial units for statistics. [Accessed Nov 2012]. Available from: http://epp.eurostat.ec.europa.eu/portal/page/portal/nuts_nomenclature/introduction
20. Marmion BP, Storm PA, Ayres JG, Semendric L, Mathews L, Winslow W, et al. Long-term persistence of Coxiella burnetii after acute primary Q fever. QJM. 2005;98(1):7-20. http://dx.doi.org/10.1093/qjmed/hci009. PMid:15625349.
21. Field PR, Mitchell JL, Santiago A, Dickeson DJ, Chan SW, Ho DW, et al. Comparison of a commercial enzyme-linked immunosorbent assay with immunofluorescence and complement fixation tests for detection of Coxiella burnetii (Q fever) immunoglobulin M. J Clin Microbiol. 2000;38(4):1645-7. PMid:10747159. PMCid:PMC86512.
22. Villumsen S, Jorgensen CS, Smith B, Uldum S, Schiellerup P, Krogfelt KA. Determination of new cutoff values for indirect immunofluorescence antibody test for Q fever diagnosis in Denmark. Diagn Microbiol Infect Dis. 2009;65(2):93-8. http://dx.doi.org/10.1016/j.diagmicrobio.2009.06.004. PMid:19748417.
23. Bacci S, Valentiner-Branth P. Mølbak K, Villumsen S, Krogfelt KA. Q feber 2006-2007. EPI-News 3. Copenhagen: Statens Seruminstitut; Jan 2009. Available from: http://www.ssi.dk/English/News/EPI-NEWS/~/media/Indhold/EN%20-%20engelsk/EPI-NEWS/2009/pdf/EPI-NEWS%20-%202009%20-%20No%203.ashx
24. Abe T, Yamaki K, Hayakawa T, Fukuda H, Ito Y, Kume H, et al. A seroepidemiological study of the risks of Q fever infection in Japanese veterinarians. Eur J Epidemiol. 2001;17(11):1029-32. http://dx.doi.org/10.1023/A:1020018907452. PMid:12380717.
25. Casolin A. Q fever in New South Wales Department of Agriculture workers. J Occup Environ Med. 1999;41(4):273-8. http://dx.doi.org/10.1097/00043764-199904000-00009. PMid:10224593.
26. Chang CC, Lin PS, Hou MY, Lin CC, Hung MN, Wu TM, et al. Identification of risk factors of Coxiella burnetii (Q fever) infection in veterinary-associated populations in southern Taiwan. Zoonoses Public Health. 2010;57(7-8):e95-101. http://dx.doi.org/10.1111/j.1863-2378.2009.01290.x. PMid:19968850.
27. Marrie TJ, Haldane EV, Faulkner RS, Kwan C, Grant B, Cook F. The importance of Coxiella burnetii as a cause of pneumonia in Nova Scotia. Can J Public Health. 1985;76(4):233-6. PMid:4052906.
28. Nowotny N, Deutz A, Fuchs K, Schuller W, Hinterdorfer F, Auer H, et al. Prevalence of swine influenza and other viral, bacterial, and parasitic zoonoses in veterinarians. J Infect Dis. 1997;176(5):1414-5. http://dx.doi.org/10.1086/517337. PMid:9359752.
29. Monno R, Fumarola L, Trerotoli P, Cavone D, Massaro T, Spinelli L, et al. Seroprevalence of Q-fever, brucellosis and leptospirosis in farmers and agricultural workers in Bari, southern Italy. Clin Microbiol Infect. 2009;15 Suppl 2:142-3. http://dx.doi.org/10.1111/j.1469-0691.2008.02151.x. PMid:19793130.
30. Schimmer B, Lenferink A, Schneeberger P, Aangenend H, Vellema P, Hautvast J, et al. Seroprevalence and risk factors for Coxiella burnetii (Q fever) seropositivity in dairy goat farmers' households in The Netherlands, 2009-2010. PLoS One. 2012;7(7):e42364. http://dx.doi.org/10.1371/journal.pone.0042364. PMid:22848762. PMCid:PMC3407076.
31. de Rooij MM, Schimmer B, Versteeg B, Schneeberger P, Berends BR, Heederik D, et al. Risk factors of Coxiella burnetii (Q fever) seropositivity in veterinary medicine students. PLoS One. 2012;7(2):e32108. http://dx.doi.org/10.1371/journal.pone.0032108. PMid:22363803. PMCid:PMC3283734.
32. Van den Brom R, Schimmer B, Schneeberger PM, Swart WA, van der Hoek W, Vellema P. Seroepidemiological Survey for Coxiella burnetii Antibodies and Associated Risk Factors in Dutch Livestock Veterinarians. PLoS One. 2013;8(1):e54021. http://dx.doi.org/10.1371/journal.pone.0054021. PMid:23342063. PMCid:PMC3546960.
33. Bacci S, Villumsen S, Valentiner-Branth P, Smith B, Krogfelt KA, Molbak K. Epidemiology and Clinical Features of Human Infection with Coxiella burnetii in Denmark During 2006-07. Zoonoses Public Health. 2012;59(1):61-8. http://dx.doi.org/10.1111/j.1863-2378.2011.01419.x. PMid:21824371.
34. Bosnjak E, Hvass AM, Villumsen S, Nielsen H. Emerging evidence for Q fever in humans in Denmark: role of contact with dairy cattle. Clin Microbiol Infect. 2010;16(8):1285-8. http://dx.doi.org/10.1111/j.1469-0691.2009.03062.x. PMid:19832723.
35. Schimmer B, Notermans DW, Harms MG, Reimerink JH, Bakker J, Schneeberger P, et al. Low seroprevalence of Q fever in The Netherlands prior to a series of large outbreaks. Epidemiol Infect. 2012;140(1):27-35. http://dx.doi.org/10.1017/S0950268811000136. PMid:21324217.
36. Whelan J, Schimmer B, Schneeberger P, Meekelenkamp J, Ijff A, van der Hoek W, et al. Q fever among culling workers, the Netherlands, 2009-2010. Emerg Infect Dis. 2011;17(9):1719-23. http://dx.doi.org/10.3201/eid1709.110051. PMid:21888803. PMCid:PMC3322078.
37. Anderson AD, Kruszon-Moran D, Loftis AD, McQuillan G, Nicholson WL, Priestley RA, et al. Seroprevalence of Q fever in the United States, 2003-2004. Am J Trop Med Hyg. 2009;81(4):691-4. http://dx.doi.org/10.4269/ajtmh.2009.09-0168. PMid:19815888.
38. van der Hoek W, Dijkstra F, Schimmer B, Schneeberger PM, Vellema P, Wijkmans C, et al. Q fever in the Netherlands: an update on the epidemiology and control measures. Euro Surveill 2010;15(12):pii=19520. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19520
39. Roest HI, Ruuls RC, Tilburg JJ, Nabuurs-Franssen MH, Klaassen CH, Vellema P, et al. Molecular epidemiology of Coxiella burnetii from ruminants in Q fever outbreak, the Netherlands. Emerg Infect Dis. 2011;17(4):668-75. http://dx.doi.org/10.3201/eid1704.101562. PMid:21470457. PMCid:PMC3377418.
40. Agerholm JS. Veterinary importance of infection with Coxiella burnetii (Q fever), the prevalence of the infection in Denmark and diagnostics. Q fever seminar by CEVA. Jan 15-16, 2012;Randers, Denmark.
41. Kantsø B, Svendsen CB, Jørgensen CS, Krogfelt KA. Comparison of two commercially available ELISA antibody test kits for detection of human antibodies against Coxiella burnetii. Scand J Infect Dis 2012;44(7):489-94. http://dx.doi.org/10.3109/00365548.2012.664777 PMid:22385345.