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Lymphogranuloma venereum: a hidden emerging problem, Barcelona, 2011

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From the beginning of 2007 until the end of 2011, 146 cases of lymphogranuloma venereum (LGV) were notified to the Barcelona Public Health Agency. Some 49% of them were diagnosed and reported in 2011, mainly in men who have sex with men. Almost half of them, 32 cases, were reported between July and September. This cluster represents the largest since 2004. This article presents the ongoing outbreak of LGV in Barcelona.

From 1 January 2007 to 30 December 2011, a total of 146 cases of lymphogranuloma venereum (LGV) were notified to the Barcelona Public Health Agency. Of those, 72 cases (49%) were diagnosed and reported in 2011. The figure shows the epidemic curve of the 139 cases who were residents of Barcelona. Of the 70 cases in 2011 who were resident in Barcelona, 31(44%) were reported between July and September.

Surveillance

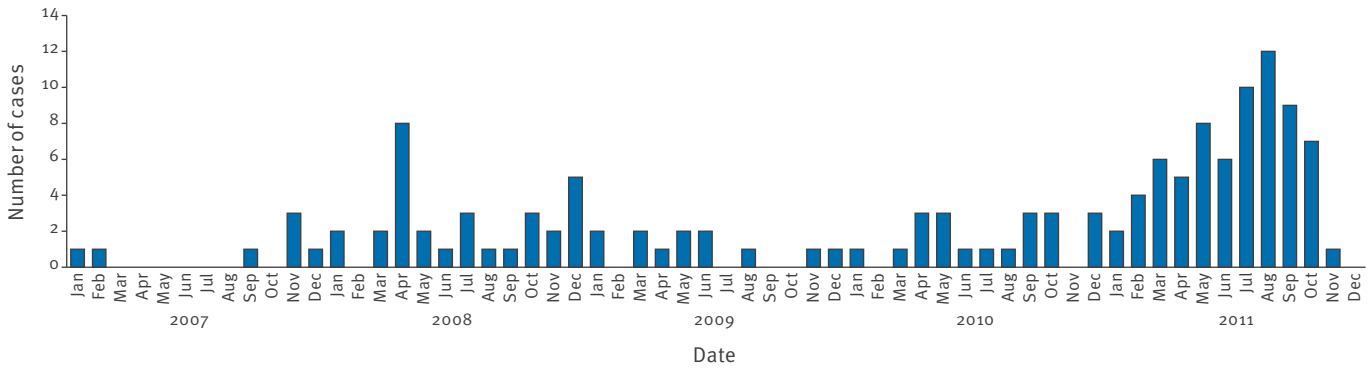
LGV surveillance in Barcelona is part of the sexually transmitted infections (STI) register, which has been active since 2007 and collects information about diagnoses in individuals tested in public or private facilities. Clinicians complete a standard data questionnaire to collect demographic, clinical and epidemiological key parameters, including date of consultation, sex, year of birth, sexual orientation, testing for human immunodeficiency virus (HIV), previous STIs, and sexual behaviour.

All data were collected by the Barcelona STI registry and were handled in a strictly confidential manner according to the requirements of the Spanish data protection Law [1].

Chlamydia trachomatis was detected by nucleic acid amplification tests. Positive samples were then confirmed with a second real-time multiplex polymerase

Figure

Cases of lymphogranuloma venereum by date of diagnosis, Barcelona residents, January 2007–December 2011 (n=139)



chain reaction that allows to differentiate serovars A-K from the L serovars [2].

Epidemiological data

After two decades without LGV notifications, a new case was diagnosed in Barcelona in 2004. It was a homosexual man who was a sexual partner of a case diagnosed in Amsterdam [3]. No further cases were detected in Barcelona until September 2007.

The median number of cases reported per month increased from two in 2010 to six in 2011. A comparison of data from the period 2007–2010 with the year 2011 showed that patients in 2011 were younger (p=0.01) and more of them had documented HIV infection (Table).

Of the 70 cases of LGV reported in 2011 that were resident in Barcelona, all were men who have sex with men (MSM), at least 66 were HIV-positive (HIV status was unknown in two cases), and 39 cases were born in Spain, 17 in South America, 12 in other countries of Western Europe and North America and one in another region. In four cases, HIV diagnosis was known at the time of the LGV diagnosis, and 22 of the cases were diagnosed with another STI in the previous 12 months. *C. trachomatis* was detected in the anal or perianal region in 67 cases, in the genital area in two cases, and for one case no data was available. Regarding the presence of symptoms, 64 cases had at least one symptom, two cases were asymptomatic, and in three cases this information was not recorded.

The time between the onset of the symptoms and the diagnosis ranged from two to 530 days, with a median of 29 days.

The mean number of new sexual partners in the 12 months before diagnosis was 26 (range: 1–100) for the 31 cases in 2011 for whom this information was obtained. Only four cases reported using a condom in the most recent sexual relationship, and three cases engaged in casual sexual intercourse while abroad. For the 27 patients whose information on location of sexual activity was available, 10 reported having had numerous sexual partners, at home or at private parties. The majority of these contacts had been established anonymously by Internet and some of them by mobile applications based on geolocation.

Control measures

To deal with the increase in LGV cases, control measures were implemented in Barcelona from September 2011: alerting STI clinics, HIV specialists and hospitals of the existence of the current outbreak of LGV; active case finding in clinical care units and microbiology laboratories; contact with patients to monitor treatment and implement partner notification; preventive

Table

Epidemiological and clinical characteristic of lymphogranuloma venereum cases, Barcelona residents, comparison of 2007–2010 with 2011 (n=139)

		2007–2010 n=69 Number (%)[a]	2011 n=70 Number (%)[a]	p value
Median age (interquartile range)		38 (34–43)	35 (29–41)	0.01
Country of birth: Spain		40 (58)	39 (56)	0.78
Sexual behaviour	MSM	64 (93)	70 (100)	
	HTS	1 (1)		
	Unknown	4 (6)		
HIV-infected	Yes	55 (80)	66 (94)	0.04
	No	8 (12)	2 (3)	
	Unknown	6 (9)	2 (3)	
Another STI diagnosed in the previous 12 months	Yes	26 (38)	22 (31)	
	No	29 (42)	23 (33)	
	Unknown	14 (20)	25 (36)	
Use of condom the last time they had sex	Yes	8 (12)	4 (6)	
	No	46 (67)	48 (69)	
	Unknown	15 (22)	18 (26)	
Contact tracing	Yes	29 (42)	42 (60)	
	No	18 (26)	9 (13)	
	Unknown	22 (32)	19 (27)	
Median of days between symptoms and diagnosis (interquartile range)		35 (14–90)	29 (13–45)	0.68
Proctitis	Yes	62 (90)	67 (96)	0.17
	No	7 (10)	3 (4)	

HIV: human immunodeficiency virus; MSM: men who have sex with men; HTS: heterosexual; STI: sexually transmitted infection.

[a] All cases were male.

activities targeting risk groups with the collaboration of non-governmental organisations.

Discussion and conclusion

This cluster represents the largest cluster of LGV cases since 2004. A previous outbreak in Barcelona, reported in 2008, had 18 cases in the course of seven months [4].

LGV is an emerging sexually transmitted infection in Europe and in North America. Occasionally, clusters of cases suggest ongoing low-level transmission in these areas [5]. However, since the first outbreak was reported in the Netherlands in 2003, new cases have been reported regularly in various European countries [6-12]. Since 2010, the United Kingdom reported an increase in cases of LGV to over 550 cases, most of them in London. The Netherlands reported 66 cases in 2010 [13,14].

Certain characteristics of LGV support the concept that it is a hidden disease: it affects vulnerable groups, is often self-treated, and misdiagnosis or delayed diagnosis is common. Early diagnosis and treatment of cases are very important because the period of communicability can vary from weeks to years, as long as active lesions are present [15].

As in other parts of Europe, the significant increase in cases of LGV in Barcelona in the last year affected the MSM population, most of them HIV-infected. The infrequent use of condoms in the last years and the high proportion of anonymous sexual contacts make this group active transmitters of STIs, including HIV. Clinicians, epidemiologists and those most susceptible to infection such as MSM, should be aware that this disease is still present in European countries, and that it could manifest in a gradual increase in cases or as outbreaks. Existing efforts to promote awareness and prevention of LGV, especially among HIV-infected patients and among physicians, should be strengthened. New technologies (e.g. Intenet, global positioning system) favour risk practices, but also provide opportunities for new prevention strategies. These new media could be used to disseminate information about preventive measures and, in the case of applications using georeferences, to facilitate the identification of contacts and tracing of patients with LGV who would benefit from timely notification. Some publications have welcomed this initiative aimed at groups of MSM who seek sexual contacts through websites [16,17]. Other experiences in STI centres, such as human sexuality seminars for MSM have proven effective in reducing risk practices in this group [18].

References

1. Ley Orgánica 15/1999, de 13 de diciembre, de Protección de Datos de Carácter Personal. [Law 15/1999 of 13 December on the protection of personal data]. Boletín Oficial del Estado BOE.. 1999;298:43088-97. Madrid: Spanish Government; 13 Dec 1999. Available from: http://www.boe.es/boe/dias/1999/12/14/pdfs/A43088-43099.pdf
2. Chen CY, Chi KH, Alexander S, Martin IM, Liu H, Ison CA, et al. The molecular diagnosis of lymphogranuloma venereum: evaluacion of a real-time multiplex polymerase chain reaction test using rectal and urethral specimens. Sex Transm Dis. 2007;34(7):451-5.
3. Vall Mayans M, Sanz Colomo B, Ossewaarde JM. First case of LGV confirmed in Barcelona. Euro Surveill. 2005;10(5):pii=2634. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=2634
4. Vall Mayans M, Caballero E, Garcia de Olalla P, Armengol P, Codina MG, Barberà MJ, et al. Outbreak of lymphogranuloma venereum among men who have sex with men in Barcelona 2007/08--an opportunity to debate sexual health at the EuroGames 2008. Euro Surveill. 2008;13(25):ppi=18908. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=18908
5. Stamm WE. Lymphogranuloma venereum. In: Holmes KK, Sparling F, Stamm WE, Piot P, Wasserheit JN, Corey L, Cohen M, editors. Sexually transmitted diseases. 4th ed. New York: Mc Graw Hill Medical; 2008. p. 595-606.
6. Nieuwenhuis RF, Ossewaarde JM, Götz HM, Dees J, Thio HB, Thomeer MG, et al. Resurgence of lymphogranuloma venereum in Western Europe: an outbreak of Chlamydia trachomatis serovar l2 proctitis in The Netherlands among men who have sex with men. Clin Infect Dis. 2004;39(7):996-1003.
7. Van de Laar MJ, Koedijk FD, Götz HM, de Vries HJ. A slow epidemic of LGV in the Netherlands in 2004 and 2005. Euro Surveill. 2006;11(9):pii=642. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=642
8. Bremer V, Meyer T, Marcus U, Hamouda O. Lymphogranuloma venereum emerging in men who have sex with men in Germany. Euro Surveill. 2006;11(9):pii=643. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=643
9. Ward H, Martin I, Macdonald N, Alexander S, Simms I, Fenton K, et al. Lymphogranuloma venereum in the United kingdom. Clin Infect Dis. 2007;44(1):26-32.
10. Herida M, de Barbeyrac B, Sednaoui P, Scieux C, Lemarchand N, Kreplak G, et al. Rectal lymphogranuloma venereum surveillance in France 2004-2005. Euro Surveill. 2006;11:(9):pii=647. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=647
11. European Centre for Disease Prevention and Control. Sexually transmitted infections in Europe 1990-2009. Stockholm: ECDC; 2011. ISBN 978-92-9193-291-7. Available from: http://ecdc.europa.eu/en/publications/Publications/110526_SUR_STI_in_Europe_1990-2009.pdf
12. Clerc M, Gallay A, Imounga L, Le Roy C, Peuchant O, Bébéar C, et al. Évolution du nombre de lymphogranulomatoses vénériennes rectales et d'infections rectales à Chlamydia trachomatis à souches non L en France, 2002-2009. [Trends of rectal lymphogranuloma venereum and rectal infection with non LGV Chlamydia trachomatis strains in France, 2002- 2009. Bulletin épidémiologique hebdomadaire. 2011;26-27-28. Available from: http://www.invs.sante.fr/Publications-et-outils/BEH-Bulletin-epidemiologique-hebdomadaire/Derniers-numeros-et-archives/Archives/2011/BEH-n-26-27-28-2011
13. Rijksinstituut voor Volksgezondheid en Milieu. Thermometer soa en hiv. [Thermometer STIs and HIV]. Bilthoven: RIVM; April 2011. Available from: http://www.soaaids.nl/documenten/rivm_thermometer2011.pdf
14. Health Protection Agency. Epidemic of Lymphogranuloma venereum (LGV) in men who have sex with men in the UK intensifies. Health Protection Report. 2011;5(24). Available from: http://www.hpa.org.uk/hpr/archives/2011/hpr2411.pdf
15. Heymann DL, editor. Control of communicable diseases manual. 19th ed. Washington: American Public Health Association; 2008.
16. Blackwell CW. Men who have sex with men and recruit bareback sex partners on the internet: implications for STI and HIV prevention and client education. Am J Mens Health. 2008;2(4):306-13.
17. Rietmeijer CA, Bull SS, McFarlane M, Patnaik JL, Douglas JM Jr. Risks and benefits of the internet for populations at risk for sexually transmitted infections (STIs): results of an STI clinic survey. Sex Transm Dis. 2003;30(1):15-9.
18. Rosser BR, Bockting WO, Rugg DL, Robinson BB, Ross MW, Bauer GR, et al. A randomized controlled intervention trial of a sexual health approach to long-term HIV risk reduction for men who have sex with men: effects of the intervention on unsafe behavior. AIDS Educ Prev. 2002;14(3 Suppl A):59-71.

First detection of *Chlamydia trachomatis* LGV biovar in the Czech Republic, 2010–2011

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We present four cases of proctitis in HIV-infected men having sex with men (MSM) living in the Czech Republic. The causative agent in all cases was the lymphogranuloma venereum (LGV) biovar of *Chlamydia trachomatis*. The spread of proctitis caused by *C. trachomatis* serovars L1–3 among MSM has been observed in several European countries, the United States and Canada since 2003. To our knowledge, no LGV cases in eastern Europe have been published to date.

Between February 2010 and February 2011, four men who have sex with men (MSM) infected with human immunodeficiency virus (HIV), who were under regular observation for HIV infection at the Bulovka University Hospital AIDS Center in Prague, Czech Republic, developed symptoms of acute proctitis. The most prominent symptom in all four patients was intensive rectal pain lasting on average 10 days (range: 7–21 days). Other symptoms included blood in the stool or pinkish mucous discharge, constipation and tenesmus. Case 1 also had one enlarged, painful inguinal lymph node. Anoscopies were performed on Case 1 and Case 3 and revealed congested, irritated mucous membranes with a whitish coating. None of the patients had urethritis, fever, or other systemic symptoms (see Table).

To our knowledge, these cases are the first LGV infections detected in the region.

Background

Lymphogranuloma venereum (LGV) is a sexually transmitted disease (STD) caused by *Chlamydia trachomatis* serovars L1–3 [1]. Rare in industrialised countries, LGV is most often restricted to Africa, Asia, South America and the Caribbean [1,2]. Outbreaks of LGV proctitis in HIV-infected MSM have, however, been reported in several European countries, the United States and Canada [3-9]. Infections with LGV serovars, mainly L2, have been reported in North America and in Belgium, Denmark, France, the Netherlands, Portugal, Spain, the United Kingdom and Sweden, but to the best of our knowledge, there have been no publications to date reporting cases in eastern Europe.

Clinical and behavioural information

Three cases were regular visitors of gay clubs where they repeatedly had protected receptive anal intercourse with casual partners, but also used sex toys without condoms. One case reported having had unprotected anal sex and used sex toys with only one partner during the year before diagnosis. The identity and possible symptoms of the partner remain unknown to us. All but one of the cases were taking combination antiretroviral therapy (cART) and their mean CD4+ T cell count was 540/µL (range: 414–602/µL). Their median age was 46 years (range: 39–47 years) and the average time since the diagnosis of HIV infection was 27.75 months (range: 9–39 months). Three of them had already been treated for one episode of STD in the past (Table).

Laboratory investigation

Rectal swabs were taken from all cases for culture and PCR for *Neisseria gonorrhoeae* and for PCR for *C. trachomatis* (Cobas CT/NG, Roche). All cases were screened serologically for syphilis. The PCR tests for *C. trachomatis* were positive in all four cases. In Case 1, PCR was also positive for *N. gonorrhoeae*. The samples positive for *C. trachomatis* were stored at -80 °C for further identification of the LGV genotype, which became available in the Czech Republic in May 2011.

The LGV genotype was identified by PCR amplification of a 262 bp fragment of target DNA using the dual-priming oligonucleotide primers (DPO) test. This method targets the *pmp-H* gene and enables simultaneous detection of LGV-serovars and differentiation of L1–3 from other serovars [10].

Treatment

Therapy with oral azithromycin 1 g once per week for three weeks was started in Case 1, who had been concomitantly diagnosed with a *N. gonorrhoeae* infection. The anorectal symptoms resolved, but the lymph node abscessed and needed to be punctured. The puncture was also PCR-positive for *C. trachomatis*. A consecutive treatment with oral doxycycline, 100 mg twice per day for five weeks, was started, with the enlarged lymph node eventually regressing after this therapy. The other three cases were treated with oral doxycycline, 100 mg twice per day for 14 to 21 days, and in all of them the symptoms resolved during the therapy. The post-treatment rectal swabs for PCR of *C. trachomatis* were negative in all four patients. The Table summarises details of the patients' risk factors, clinical symptoms and therapy.

Table. Risk factors, clinical symptoms, therapy and sexually transmitted disease history of lymphogranuloma venereum cases, Czech Republic, February 2010 to February 2011 (n=4)

Discussion and conclusions

The Czech cases of LGV infection were very similar to the cases reported both in North America and western Europe [4]. All cases were HIV-infected MSM who used sex toys; three of them had had numerous sexual contacts. Furthermore, the clinical symptoms were very similar and their intensity corresponded to what is typical for LGV proctitis [11]. Although the method we used to identify LGV DNA cannot differentiate between L1, L2 and L3 genotypes, it distinguishes L1–3 from other serovars; the presence of the LGV infections in the region of eastern Europe is therefore evident.

The recommended therapy for LGV proctitis is oral doxycycline, 100 mg orally twice per day for three weeks [12]. Two of our cases were treated with the recommended dose of doxycycline, but only for two weeks. This shorter regimen was chosen because the LGV aetiology was not known, as the method for the identification of LGV biovars was introduced in the Czech Republic in May 2011. Nevertheless, even the two-week therapy with doxycycline proved effective enough in our cases.

The increased frequency of identification of LGV serotypes of *C. trachomatis* in developed countries in recent years is certainly connected to the introduction of modern molecular diagnostic methods into routine practice; on the other hand, it also closely correlates with the rapid increase in the incidence of syphilis among MSM in the same regions, including the Czech Republic [13-15]. This situation probably demonstrates decreasing awareness on the part of MSM about the risk of transmission of STDs. The frequent use of sex toys among patients with LGV proctitis indicates that these objects may play an important role in the transmission of LGV biovars of *C. trachomatis* [16,17].

This new epidemiological situation requires thorough analysis in order to adapt interventional strategies especially for population groups at particular risk such as HIV-infected MSM. Active case-finding and contact tracing for LGV infection should be included in routine healthcare for such high-risk populations.

In addition, the cases described here document that the spread of LGV strains of *C. trachomatis* has reached eastern Europe, and further reports of the identification of this pathogen in this region can be expected soon after the introduction of appropriate diagnostic methods in this region.

Table

Risk factors, clinical symptoms, therapy and sexually transmitted disease history of lymphogranuloma venereum cases, Czech Republic, February 2010 to February 2011 (n=4)

Case	Risk factors	Symptoms	Therapy	Other STDs	cART
1	Protected sexual intercourse with multiple sexual partners, use of sex toys	Rectal pain, constipation, blood in stool, tenesmus, unilateral inguinal painful lymphadenopathy	Azithromycin (1 g orally every five days for three weeks); doxycycline (100 mg twice per day for five weeks)	Coinfection with *N. gonorrhoeae*, syphilis in anamnesis	Lopinavir/ritonavir + zidovudin + lamivudin
2	Unprotected sexual intercourse with a stable partner, use of sex toys	Rectal pain, constipation, mucous discharge with blood, tenesmus	Doxycycline (100 mg orally twice per day for two weeks)	Syphilis in anamnesis	Tenofovir + zidovudin + lamivudin
3	Protected sexual intercourse with multiple sexual partners, use of sex toys	Mucous pinkish stool, constipation, tenesmus	Doxycycline (100 mg orally twice per day for two weeks)	No	Lopinavir/ritonavir + tenofovir + emtricitabine
4	Protected sexual intercourse with multiple sexual partners, use of sex toys	Rectal pain, mucous pinkish stool, constipation, tenesmus	Doxycycline (100 mg orally twice per day for three weeks)	Gonorrhoea in anamnesis	No

cART: combination antiretroviral therapy; STD: sexually transmitted disease.

References

1. Mabey D, Peeling RW. Lymphogranuloma venereum. Sex Transm Infect. 2002;78(2):90-2.
2. Blank S, Schillinger JA, Harbatkin D. Lymphogranuloma venereum in the industrialised world. Lancet. 2005;365(9471):1607-8.
3. Kropp RY, Wong T; Canadian LGV Working Group. Emergence of lymphogranuloma venereum in Canada. CMAJ. 2005;172(13):1674-6.
4. Savage EJ, van de Laar MJ, Gallay A, van der Sande M, Hamouda O, Sasse A, et al. Lymphogranuloma venereum in Europe, 2003-2008. Euro Surveill. 2009;14(48):pii=19428. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19428
5. Nieuwenhuis RF, Ossewaarde JM, Gotz HM, Dees J, Thio HB, Thomeer MG, et al. Resurgence of lymphogranuloma venereum in Western Europe: an outbreak of Chlamydia trachomatis serovar L2 proctitis in The Netherlands among men who have sex with men. Clin Infect Dis. 2004;39(7):996-1003.
6. Ward H, Martin I, Macdonald N, Alexander S, Simms I, Fenton K, et al.: Lymphogranuloma venereum in the United Kingdom. Clin Infect Dis. 2007;44(1):26-32.
7. Herida M, de Barbeyrac B, Sednaoui P, Scieux C, Lemarchand N, Kreplak G, et al.: Rectal lymphogranuloma venereum surveillance in France 2004-2005. Euro Surveill 2006;11(9):pii=647. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=647
8. Castro R, Baptista T, Vale A, Nunes H, Prieto E, Mansinho K, et al. Anorectal lymphogranuloma venereum: the first two confirmed cases in Portugal. Euro Surveill. 2008;13(50):pii=19060. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19060
9. Cusini M, Boneschi V, Tanzi C, Girgenti V, De Vries H, Alessi E. Ano-rectal lymphogranuloma venereum: the first case in Italy. G Ital Dermatol Venereol. 2008;143(1):83-5.
10. Chen CY, Chi KH, Alexander S, Martin IM, Liu H, Ison CA, et al. The molecular diagnosis of lymphogranuloma venereum: evaluation of a real-time multiplex polymerase chain reaction test using rectal and urethral specimens. Sex Transm Dis. 2006;34:451-455.
11. Hoie S, Knudsen LS, Gerstoft J. Lymphogranuloma venereum proctitis: a differential diagnose to inflammatory bowel disease. Scand J Gastroenterol. 2011;46(4):503-10.
12. McLean CA, Stoner BP, Workowski KA. Treatment of lymphogranuloma venereum. Clin Infect Dis. 2007;44(Suppl 3):S147-52.
13. Savage EJ, Hughes G, Ison C, Lowndes CM. Syphilis and gonorrhoea in men who have sex with men: a European overview. Euro Surveill. 2009;14(47):pii=19417. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19417
14. Zákoucká H, Polanecký V, Kastánková V. Syphilis and gonorrhoea in the Czech Republic. Euro Surveill. 2004;9(12):pii=496. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=496
15. Kuklová I, Kojanová M, Zákoucká H, Pánková R, Velcevský P, Rozehnalová Z, et al. Dermatovenereology in the post-communist era: syphilis in Prague during 1999 to 2005. Dermatol Clin. 2008;26(2):231-7.
16. O'Connor C, O'Connor MB, Clancy J, Ryan A. Sex toy hygiene. Int J STD AIDS. 2009;20(11):806-7.
17. Ronn MM, Ward H. The association between lymphogranuloma venereum and HIV among men who have sex with men: systematic review and meta-analysis. BMC Infect Dis 2011;11:70. doi: 10.1186/1471-2334-11-70.

Influenza A(H1N1)pdm09 antibodies after pandemic and trivalent seasonal influenza vaccination as well as natural infection in November 2010 in Hamburg, Germany

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The 2009 influenza pandemic has introduced the new re-assorted influenza A(H1N1)pdm09 virus which recirculated during the 2010/11 influenza season. Before that season, it was possible to acquire protective immunity either by pandemic or seasonal influenza vaccination against influenza A(H1N1)pdm09 or by natural infection. To obtain data on vaccination coverage and antibody levels in a reference population and to calculate whether or not the herd immunity threshold (HIT, calculated as 33% given an R_0 of 1.5) was reached at the beginning of the 2010/11 season we performed a seroprevalence study in November 2010 in Hamburg, Germany. Antibody titres were assessed applying a haemagglutination inhibition test. Vaccination coverage was very low: 14% for pandemic and 11% for seasonal 2010/11 vaccinations. Even in those with underlying risk factors, vaccination coverage was not much higher: 17% for both vaccines. Serological analysis revealed antibody titres of ≥1:10 in 135 of 352 (38%) and of ≥1:40 in 61 of 352 study participants (17%). Specific antibodies were measurable in 26% of those without history of vaccination or natural infection, indicating a high proportion of subclinical and mild influenza disease. Nevertheless, the HIT was not reached, leaving the majority of the population susceptible to influenza A(H1N1)pdm09 and its potential complications.

Introduction

In April 2009, a new re-assorted influenza A virus emerged causing the influenza A(H1N1)pdm09 pandemic [1]. In Germany, a monovalent AS03-adjuvanted vaccine against the pandemic influenza A(H1N1)pdm09 (Pandemrix) has been available since October 2009 and was recommended to persons at risk for severe disease but was also offered to anyone who wanted to be vaccinated for maximal personal protection. In addition, a trivalent seasonal influenza vaccine was available as in previous years [2]. The end of the pandemic was declared in August 2010, and haemagglutinin and neuraminidase antigens H1 and N1 of the pandemic strain were integrated into the 2010/11 trivalent seasonal influenza vaccines, which became available in Germany in September 2010. Therefore, antibodies to influenza A(H1N1)pdm09 detected in November 2010, before the start of the influenza season 2010/11 were a consequence either of vaccination with the pandemic or the 2010/11 seasonal vaccine or of natural infection.

It was discussed before the 2010/11 influenza season, whether or not this unique situation would lead to high immunity in the population and thus to a particularly mild influenza season, as it was generally assumed that the influenza A(H1N1)pdm09 virus would remain the predominant virus strain in the 2010/11 season. The proportion of a population that must be immune to reduce the mean number of secondary infections per infectious individual to less than one, is called the herd immunity threshold (HIT). The HIT indicates that a certain level of population immunity reduces the probability of infection of non-immune individuals. In viral diseases, this particular immunity threshold directly depends on the transmission potential of the infectious agent. Direct and indirect protection reduce the reproduction rate, eventually stopping or preventing an epidemic wave [3].

In addition to vaccine effectiveness and duration of protection, the degree of vaccine uptake in the population during a mass vaccination campaign is essential for the mitigation of pandemic influenza. In Germany and other countries, there was a significant media-driven debate on risks and benefits associated with adjuvanted and non-adjuvanted pandemic influenza vaccines

[4]. As a result, vaccination coverage remained low in Germany, in the general population and in risk groups [5].

In a seroprevalence study in the second largest city in Germany, we intended (i) to assess the proportion of persons with detectable antibodies against influenza A(H1N1)pdm09 and to estimate whether or not the HIT was reached, (ii) to compare antibody titres in vaccinated and previously infected persons in general and in high risk subgroups, and (iii) to obtain information on the acceptance of past pandemic vaccination campaigns and pandemic vaccines in a reference population.

Methods

Questionnaire

We performed a cross-sectional survey in Hamburg between 1 and 21 November 2010. Hamburg is the second largest city in Germany with 1.8 million inhabitants. The recruitment period was kept short to allow as many seasonal influenza vaccinations as possible to happen prior to enrolment, while excluding potential natural infections that might occur at the beginning 2010/11 influenza season. Volunteers were recruited through advertisements in the city's public transportation system. Registration for participation in the survey was possible via telephone or internet. Besides age above 18 years and ability to understand the informed consent process there were no particular in- or exclusion criteria. Basic demographic data, information on influenza-like illness, vaccination status as well as concomitant diseases or risk factors for complicated influenza were obtained using a standardised questionnaire after having obtained written consent. Each questionnaire was reviewed for completeness and consistency by a trained member of the investigation team together with the participant. Questions included whether or not the participant had received the regular seasonal influenza vaccine after April 2009 and/or the pandemic vaccine. A condition 'past influenza A(H1N1) disease' (referred to as 'natural infection' in this manuscript) was assumed if the participant reported history of influenza diagnosed by a medical doctor or treatment with neuraminidase inhibitors prescribed by a medical doctor since April 2009. Questions addressing the participants' opinion on the past vaccination campaigns as well as pandemic vaccines as such included whether or not the participant had general concerns with respect to adjuvants contained in vaccines and if yes, whether he/she would still be willing to receive adjuvanted vaccines in future pandemics.

Ethical approval was obtained from the local ethics committee of the Hamburg chamber of physicians.

Virological analysis

A serum sample was obtained from each participant, centrifuged at 2,000 g for 10 minutes, and stored at -80 °C until further processing. The samples were analysed for antibodies against influenza A(H1N1)pdm09 virus by an in-house haemagglutination inhibition (HI) test which gave clear and highly reproducible results and allowed to determine titres of 1:10 against influenza A(H1N1)pdm09 virus (sensitivity 1.0 and specificity 0.96). This in-house HI test has been described in detail elsewhere [6]. In brief, the HI-test was designed to reach a high specificity without losing relevant sensitivity and could show that there was no cross-reactivity between antibodies against seasonal influenza A/Brisbane/59/2007(H1N1) and antibodies against influenza A(H1N1)pdm09 influenza (unpublished data). The external reference serum pool obtained from the National Institute for Biological Standards and Control (NIBSC), with a defined titre after multi-laboratory testing of 1:160, however, was reproducibly equivalent to a titre of 1:80 (i.e. one two-fold dilution lower) in our test.

Statistical analysis

Statistical analyses were performed from anonymised data by members of the study team who had not been involved in the recruitment and questionnaire procedures. For calculating the HIT, we used a basic reproduction number (R_0) of 1.5 which was assumed to be the most realistic by the European Centre for Disease Prevention and Control (ECDC) [7]. This R_0 lies within a range between 1.2 and 1.7, which has been reported for Europe and the United States of America [8,9]. Using the simplified equation to calculate the HIT=$(R_0-1)/R_0$ [10], protective antibody titres would have been necessary in at least 33% (17–41%) of the population to prevent a significant influenza wave in the 2010/11 season.

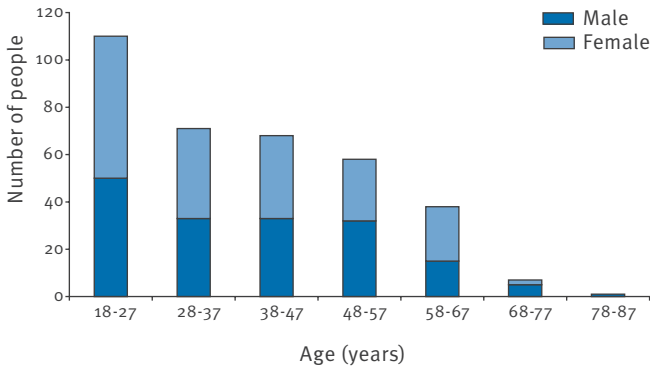
Results

Of the 353 study persons, 169 (48%) were male. Median age was 39 years (range 18–78), see Figure. Co-morbidities including diabetes, cancer, immunosuppression, and chronic liver or kidney disease were present in 52 of 353 individuals (15%).

Vaccination coverage for pandemic (49/353, 14%) and 2010/11 seasonal (40/353, 11%) influenza was very low. Ten participants (2.8%) reported to have been

Figure

Study population, stratified by age and sex, Hamburg, 1–21 November 2010 (n=353)



diagnosed with influenza (n=2) or to have been treated with neuraminidase inhibitors (n=8) since April 2009 and were, hence, classified as probable 'natural infections'. Seventy-nine individuals (22%) reported evidence for at least one of the three options, pandemic vaccination, seasonal 2010/11 vaccination or natural infection, for acquisition of specific antibodies. Of the 52 persons with co-morbidities, nine stated to have received the pandemic vaccine and a further nine stated to have received the 2010/11 seasonal influenza vaccine. Four had been vaccinated with both vaccines.

Serological analysis of the entire study population revealed influenzaA(H1N1)pdm09 antibody titres of ≥1:10 in 135 of 352 (38%) and of ≥1:40 in 61 of 352 (17%) samples (Table 1), which is below the calculated HIT of 33% for the ≥1:40 levels. A titre of ≥1:20 (which represents a titre of ≥1:40 in the external NIBSC-reference serum pool and is often deemed as protective) was reached by 97 of 352 (28%).

Proportions of subjects with antibody titres in the three subgroups (i) pandemic vaccination, (ii) seasonal influenza 2010/11 vaccination, and (iii) 'natural infection' are shown in Table 1 for titres ≥1:10 and ≥1:40. Among pandemic vaccine recipients, antibody titres ≥1:10 were detected in 39 of 49 and titres ≥1:40 in 23 of 49 people one year (range 8–14 months) after vaccination with the pandemic vaccine. Similar proportions of antibody titres of ≥1:10 and ≥1:40 were detected in those that had received the 2010/11 seasonal influenza vaccine shortly (0–10 weeks) before participating in this study: 36 of 40 vaccine recipients had antibody titres ≥1:10, 22 of 40 had titres ≥1:40. Among the 14 individuals who were sequentially immunised with both the pandemic and seasonal influenza 2010/11 vaccines, 13 exhibited titres ≥1:10 and nine titres ≥1:40. Interestingly, antibody titres ≥1:10 were detected in 71 of 272 (26%) of individuals without any history of either disease or vaccination (24/272 (9%) for titres ≥1:40), indicating asymptomatic infection in about a quarter of all participants (Table 1). Yet the proportion of individuals with positive antibody titres was significantly higher in those with a history of vaccination and/or disease than in those without for both titres, ≥1:10 (80% versus 26%, $P<0.0001$) and ≥1:40 (47% versus 9%, $P<0.0001$).

Overall, the proportion of vaccinated individuals with high antibody levels (≥1:40) decreased with age in all subgroups while such a trend was not obvious for those with antibody levels ≥1:10 (Table 1). Proportions of subjects with titres ≥1:10 and ≥1:40 were comparable in those with and without co-morbidities (≥1:10 titres: 46% versus 37%, $P=0.2$; ≥1:40 titres: 15% versus 18%, $P=0.7$).

When asked about their personal opinion about past pandemic vaccination strategies, 58% of the participants stated that mass vaccinations and respective campaigns in 2009 were not justified in retrospect. However, 63% would accept to receive pandemic vaccinations in future pandemic situations. Twenty-two percent (76/353) stated to have concerns regarding the adjuvant that was included in the pandemic vaccine. Yet 61% (46/76) of them would be open for future pandemic vaccinations despite their adjuvant-related concerns (Table 2).

Table 1

Antibody titres against influenza A(H1N1)pdm09 in the study population, stratified by age group and antibody level, after vaccination or natural infection, Hamburg, Germany, 1–21 November 2010 (n=353)

Study participants		18–39 years	40–59 years	≥60 years	All
Recipients of pandemic vaccination	Number (%)	26/193 (13)	15/125 (12)	8/35 (23)	49/353 (14)
HI-test results	Titre ≥1:10 (%)	21/26 (81)	10/15 (67)	8/8 (100)	39/49 (80)
	Titre ≥1:40 (%)	15/26 (58)	6/15 (40)	2/8 (25)	23/49 (47)
Recipients of 2010/11 seasonal vaccination	Number (%)	15/193 (8)	15/125 (12)	10/35 (29)	40/353 (11)
HI-test results	Titre ≥1:10 (%)	15/15 (100)	11/15 (73)	10/10 (100)	36/40 (90)
	Titre ≥1:40 (%)	11/15 (73)	9/15 (60)	2/10 (20)	22/40 (55)
Natural infection[a]	Number (%)	5/193 (3)	3/125 (2)	2/35 (6)	10/353 (3)
HI-test results	Titre ≥1:10 (%)	3/5 (60)	1/3 (33)	2/2 (100)	6/10 (60)
	Titre ≥1:40 (%)	2/5 (40)	0/3 (0)	0/2 (0)	2/10 (20)
No evidence for natural infection[a] or vaccination	Number (%)	154/193 (80)	99/125 (79)	20/35 (57)	273/353 (77)
HI-test results	Titre ≥1:10 (%)	53/154 (34)	15/99 (15)	3/19 (15)	71/272 (26)
	Titre ≥1:40 (%)	17/154 (11)	6/99 (6)	1/19 (1)	24/272 (9)
All	Number	193	125	35	353
HI-test results	Titre ≥1:10 (%)	86/193 (45)	31/125 (25)	18/34 (53)	135/352 (38)
	Titre ≥1:40 (%)	41/193 (21)	16/125 (13)	4/34 (12)	61/352 (17)

HI: haemagglutination inhibition.

[a] 'Natural infection' was defined as influenza disease diagnosed by a physician or treatment with neuraminidase inhibitors since April 2009.

Discussion

In the present study, the calculated HIT was not met for protective antibody levels as neither a protective titre of ≥1:40 nor a titre of ≥1:20 was reached by 33% of the study population. Approximately one third of all participants showed measurable antibody titres of ≥1:10 but this titre lies below the protective level (see below). This reflects a rather low proportion with protective or even detectable antibody levels in the study population about one and a half years after the pandemic and roughly one year after pandemic mass vaccinations in Hamburg, Germany. The true proportion of people with measurable antibody titres in the general population is likely to be even lower than the numbers presented here due to potential recruitment bias in our study population: the participants may have had a more positive view on influenza vaccinations than the general population.

When this study was conducted in November 2010 just before the beginning of the 2010/11 influenza season, antibodies against influenza A(H1N1)pdm09 could be detected in the majority of those who had received pandemic vaccination in late 2009 as well as also in those who had received seasonal 2010/11 influenza vaccination recently. While during the pandemic almost exclusively the adjuvanted influenza vaccine had been used in Germany, almost all trivalent influenza vaccines used in the 2010/11 season were non-adjuvanted vaccines. Assuming that immunity lasts longer after administration of adjuvanted vaccines (unpublished results), this might explain why the prevalence of ≥1:10 and ≥1:40 antibody titres was similar between the two groups of vaccine recipients even though the pandemic vaccine was administered one year earlier. However, in some study participants the determination of antibodies may have been performed too soon after vaccination to detect high antibody titres induced by the 2010/11 seasonal vaccine. Interestingly, a high proportion of individuals without history of vaccination or infection had detectable antibody levels. This indicates a high rate of infections with a subclinical or mild clinical course in the study population, which is in line with other seroprevalence studies [11-14]. In a recently published report by von Kries et al., who compared influenza A(H1N1)pdm09-specific antibody titres in unvaccinated children in Germany before and after the pandemic, the serologically determined incidence of pandemic influenza was as high as 25.4% in the age group of 1–4 year-olds and 28% in children aged 5–17 [6].

Overall, the proportion of vaccinated individuals with high antibody levels (≥1:40) decreased with age in all subgroups possibly reflecting declining immune response with age. However, this potentially waning immune response in the elderly was not seen at the ≥1:10 antibody level in the vaccinated subgroups possibly indicating that vaccination confers better immune response than asymptomatic infection. Vaccination coverage was also low in participants with co-morbidities generally accepted to confer a high risk for complicated influenza disease [1], and antibody levels in this group were not significantly higher compared to antibody levels in individuals without risk factors. This implies that immune protection of the sub-population with risk factors may have been insufficient before the past 2010/11 influenza season.

It is not fully established how well HI antibody titres reported in the literature correlate with protection from disease, but generally a HI-titre of ≥1:40 is regarded to indicate protection [15]. The in-house HI test used in this study was designed to be highly specific, which resulted in a two-fold lower titre when testing the external reference serum pool obtained from the NIBSC. Consequently, titres of 1:20 are equivalent to 1:40 titres obtained by less stringent HI-tests [6]. On the other hand, recently published preliminary data from the Influenza Monitoring Vaccine Effectiveness in Europe (I-MOVE) network indicate low vaccine effectiveness of the seasonal 2010/11 influenza vaccine [16,17]. Data from the United Kingdom, for example, have shown a vaccine effectiveness of the pandemic and the 2010/11 seasonal influenza vaccines against RT-PCR-confirmed influenza in the 2009/10 and 2010/11 seasons of 34% and 46%, respectively, and of 63% in those who were vaccinated in both seasons [18]. In an accompanying editorial, Puig-Barbera hypothesised the test-negative study design of the observational study or adjustments

Table 2

Vaccination history of the study participants, their concerns related to the adjuvanted pandemic vaccine and their willingness to receive pandemic vaccination in the future, Hamburg, Germany, 1–21 November 2010 (n=353)

Question	Answer	All	Male (n=169)	Female (n=184)
Have you received 2009/10 seasonal influenza vaccination?	Yes	82 (23%)	45 (27%)	37 (20%)
Are you planning 2010/11 seasonal influenza vaccination?	Yes	75 (21%)	37 (22%)	38 (21%)
Have you ever been vaccinated against seasonal influenza?	Yes	182 (52%)	91 (54%)	91 (49%)
Do you have concerns regarding adjuvants used in the pandemic vaccine?	Yes	76 (22%)	31 (18%)	45 (24%)
Would you agree to receive pandemic vaccination in the future?	Yes (all)	206 (58%)	93 (55%)	113 (61%)
	Yes (those concerned about adjuvants)	46/76 (61%)	20/31 (65%)	26/44 (59%)

for some variables in the analyses might have led to an underestimation of the vaccine effectiveness [19]. When applying an arbitrary cut-off titre of ≥1:40, our data are in line with the observed low vaccine effectiveness. Nevertheless, influenza A(H1N1)pdm09 antibodies were significantly more often detected in those who had received pandemic or seasonal vaccination than in those that had not. Interestingly, in our study the small number of participants who had been vaccinated with both the pandemic and the seasonal vaccine exhibited highest antibody titres of ≥1:10 in 93% and ≥1:40 in 64%, possibly suggesting a prime-boost effect.

Vaccination coverage was very low in our study population. In Sweden, vaccination coverage with the same pandemic vaccine was higher than 60% in the general population and higher than 95% in healthcare workers [20]. According to a representative telephone survey, vaccination coverage in Hamburg after the influenza A(H1N1)pdm09 vaccination campaign was as low as 8.4% (95% confidence interval: 4.9–14.0) [12]. The fact that the vaccination coverage in our study population was higher than in the telephone survey suggests our study sample may have suffered from a recruitment bias as discussed above. Nevertheless, as other studies also found low vaccination coverage for pandemic influenza in Hamburg and other German regions [4], it is reasonable to assume that our data on vaccine-induced immunity can be generalised for the entire German population.

In Germany, there was a vivid media-driven debate on risks and benefits associated with the mass vaccination campaigns and the adjuvant containing squalene used in pandemic vaccines [4,5]. A high proportion of participants stated to have had reservations regarding the adjuvant and the usefulness of the mass vaccination campaigns. Nevertheless, concerns related to the adjuvant did not influence the participants' willingness to receive pandemic vaccination in the future. This could be interpreted to indicate that the low vaccination coverage in our study population (and possibly in all of Germany) was related to personal distrust of mass vaccination campaigns and adjuvants in general rather than of the effectiveness of the pandemic vaccine. This information could be important for planning health protection measures in future pandemics in that it may be important to provide more detailed information on the vaccine and to justify both active and non-active ingredients, i.e. the form of the active ingredient (whole virion versus split/subunit vaccines), the adjuvant as well as non-active excipients.

The present study has several limitations. In addition to the recruitment bias, the way this study was conducted includes the risk of recall bias in that some participants may not exactly remember the type and date of vaccinations received one and a half years before the study or may be unable to provide reliable information on influenza-like illness. We do not think, however, that this had a significant impact on our major findings as the study participants are likely to remember precisely whether or not they have received pandemic vaccination because of the particular circumstances around the pandemic vaccinations (such as information, specific locations or waiting lists), while seasonal 2010/11 vaccination has only been available for a few weeks prior to this study. Furthermore, information bias may have been introduced by imprecise definitions; for example, the definition of 'natural infection' may not reflect true influenza infection due to the unspecific disease symptoms and the fact that influenza disease is usually not confirmed by laboratory testing. We are also aware of the fact that the exclusion of children and adolescents limit our findings because these groups are at high risk for influenza A(H1N1)pdm09 infection and important for its transmission [6]. While the sex distribution in the study population resembled the general population in Hamburg and Germany overall, the age distribution differed in that a comparatively high number was recruited in the younger age groups while the elderly population was underrepresented [21]. Nevertheless, the population recruited for this survey through advertisements in the public transport system represented a relevant population with respect to influenza transmission that was representative for the city of Hamburg as well as other regions in Germany. Finally, a few persons with positive antibody titres may have been missed by using the in-house HI test alone without adding the sensitivity of a second test (i.e. a microneutralisation test), but we think that a slight increase in sensitivity would have had a negligible influence on the results [15].

In conclusion, the HIT for influenza A(H1N1)pdm09 had not been reached in Hamburg, Germany by November 2010, leaving the majority of the population susceptible to the infection and its potential complications, although everyone had the chance to acquire specific immunity either during the mass vaccination campaign in 2009 (which targeted the total population in Germany), during routine seasonal influenza vaccination in 2010 (targeting specific risk groups) or by natural infection. Our data confirm the view that vaccination and potentially re-vaccination with influenza A(H1N1) pdm09-containing vaccines in 2009/10 and 2010/11 induced measurable specific antibodies. It is important to increase the proportion of immune persons in a population above the HIT with effective and fast acting vaccines especially in a pandemic situation characterised by a general lack of pre-existing specific immunity. While protective antibody titres against influenza viruses have been well defined for the purpose of assessing immunogenicity of vaccines, their correlation with clinical protection especially with regard to long-term protection is less clear. Therefore it seems important to validate the currently used serological correlates of protection against influenza viruses [15] and to continue the monitoring of breakthrough infections in observational studies during future influenza seasons as it has been done by I-MOVE.

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References

1. Writing Committee of the WHO Consultation on Clinical Aspects of Pandemic (H1N1) 2009 Influenza, Bautista E, Chotpitayasunondh T, Gao Z, Harper SA, Shaw M, et al. Clinical aspects of pandemic 2009 influenza A (H1N1) virus infection. N Engl J Med. 2010;362(18):1708-19.
2. Robert Koch Institute. Impfung gegen die Neue Influenza A (H1N1). [Vaccination against the New Influena A (H1N1)]. Epidemiologisches Bulletin 41/2009. German. Available from: http://www.rki.de/cln_169/nn_1378492/DE/Content/Infekt/EpidBull/Archiv/2009/41__09,templateId=raw,property=publicationFile.pdf/41_09.pdf
3. Georgette NT. Predicting the herd immunity threshold during an outbreak: a recursive approach. PLoS One. 2009;4(1):e4168.
4. Schmidt F, Tempel G. Repräsentative telefonische Erhebung zur Impfung gegen die pandemische Influenza (H1N1) 2009. [Representative telephone survey on vaccination coverage against pandemic influenza (H1N1) 2009. Epidemiologisches Bulletin 25/2010. German. Available from: http://www.rki.de/cln_226/nn_1759378/DE/Content/Infekt/EpidBull/Archiv/2010/25__10,templateId=raw,property=publicationFile.pdf/25_10.pdf
5. Walter D, Böhmer MM, Heiden M, Reiter S, Krause G, Wichmann O. Monitoring pandemic influenza A(H1N1) vaccination coverage in Germany 2009/10 - results from thirteen consecutive cross-sectional surveys. Vaccine. 2011;29(23):4008-12.
6. von Kries R, Weiss S, Falkenhorst G, Wirth S, Kaiser P, Huppertz HI, et al. Post-Pandemic Seroprevalence of Pandemic Influenza A (H1N1) 2009 Infection (Swine Flu) among Children <18 Years in Germany. PLoS One. 2011;6(9):e23955.
7. European Centre for Disease Prevention and Control (ECDC). Understanding the Reproduction Number and Generation Times of Influenza A(H1N1) 2009. Stockholm: ECDC; 21 Aug 2009. Available from: http://www.ecdc.europa.eu/en/activities/sciadvice/Lists/ECDC%20Reviews/ECDC_DispForm.aspx?List=512ff74f%2D77d4%2D4ad8%2Db6d6%2Dbfof23083f30&ID=641
8. Department of Health. Swine Flu. UK Planning Assumptions. London: Department of Health; 16 Jul 2009. Available from: http://www.dh.gov.uk/prod_consum_dh/groups/dh_digitalassets/documents/digitalasset/dh_102891.pdf
9. White LF, Wallinga J, Finelli L, Reed C, Riley S, Lipsitch M, et al. Estimation of the reproductive number and the serial interval in early phase of the 2009 influenza A/H1N1 pandemic in the USA. Influenza Other Respi Viruses. 2009;3(6):267-76.
10. Grassly NC, Fraser C. Mathematical models of infectious disease transmission. Nat Rev Microbiol. 2008;6(6):477-87.
11. Seroepidemiological studies of pandemic influenza A (H1N1) 2009 virus. Wkly Epidemiol Rec. 2010;85(24):229-35.
12. Miller E, Hoschler K, Hardelid P, Stanford E, Andrews N, Zambon M. Incidence of 2009 pandemic influenza A H1N1 infection in England: a cross-sectional serological study. Lancet. 2010;375(9720):1100-8.
13. Bandaranayake D, Huang S, Bissielo A, Wood T. Seroprevalence of the 2009 influenza A (H1N1) pandemic in New Zealand. Wellington: Ministry of Health: May 2010. Available from: http://www.health.govt.nz/system/files/documents/publications/seroprevalence-flu-2009.pdf
14. Hardelid P, Andrews NJ, Hoschler K, Stanford E, Baguelin M, Waight PA, et al. Assessment of baseline age-specific antibody prevalence and incidence of infection to novel influenza A/H1N1 2009. Health Technol Assess. 2010 Dec;14(55):115-92.
15. Allwinn R, Geiler J, Berger A, Cinatl J, Doerr HW. Determination of serum antibodies against swine-origin influenza A virus H1N1/09 by immunofluorescence, haemagglutination inhibition, and by neutralization tests: how is the prevalence rate of protecting antibodies in humans? Med Microbiol Immunol. 2010;199(2):117-21.
16. Kissling E, Valenciano M, I-MOVE case-control studies team. Early estimates of seasonal influenza vaccine effectiveness in Europe, 2010/11: I-MOVE, a multicentre case-control study. Euro Surveill. 2011;16(11):pii=19818. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19818
17. Savulescu C, Jiménez-Jorge S, de Mateo S, Ledesma J, Pozo F, Casas I, et al. Effectiveness of the 2010/11 seasonal trivalent influenza vaccine in Spain: preliminary results of a case-control study. Euro Surveill. 2011;16(11).:pii=19820. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19820
18. Pebody R, Hardelid P, Fleming DM, McMenamin J, Andrews N, Robertson C, et al. Effectiveness of seasonal 2010/11 and pandemic influenza A(H1N1)2009 vaccines in preventing influenza infection in the United Kingdom: mid-season analysis 2010/11. Euro Surveill. 2011;16(6):pii=19791. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19791
19. Puig-Barberà J. 2010-2011 influenza seasonal vaccine, preliminary mid-season effectiveness estimates: reason for concern, confounding or are we following the right track? Euro Surveill. 2011;16(11):pii=19821. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19821
20. Ortqvist A. Challenges for implementation of the pandemic vaccination program: the Swedish experience. 1st International Conference on Controversies in Vaccination in Adults; 27-30 Jan 2011, Berlin, Germany.
21. Die soziale Situation in Deutschland. Bevölkerungsentwicklung und Altersstruktur. [The social situation in Germany. Demographic development and age structure]. Bonn: Bundeszentrale für Politische Bildung. [Accessed 22 Sep 2011. Available from: http://www.bpb.de/wissen/1KNBKW,0,0,Bev%F6lkerungsentwicklung_und_Altersstruktur.html

Assessment of public health issues of migrants at the Greek-Turkish border, April 2011

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A joint mission to assess the public health situation of migrants in Greek detention centres was undertaken in April 2011 by the European Centre for Disease Prevention and Control (ECDC) and the World Health Organization (WHO) Regional Office for Europe. The assessment visit follows the increased migration to the Evros prefecture, Eastern Macedonia and Thrace region, at the Greek-Turkish border where large numbers of migrants are entering Greece via the Evros River, a natural border. Migrants are housed in local detention centres. The main problem in detention centres are the substandard hygiene conditions, especially overcrowding and lack of personal hygiene facilities, lack of basic supplies and lack of access to fresh air and physical exercise. As the migration route via the Evros region is increasingly used since 2009, and due to the unstable political situation in North Africa and the Middle East, an increased influx of migrants was to be expected with the falling water levels of the Evros River in summer, resulting in further deterioration of the already critical situation in the Thrace region's detention centres.

Background

Since the beginning of 2010, the number of migrants [1] that enter the Evros prefecture by crossing the Greek-Turkish border has increased considerably. Until the end of 2009, approximately 3,500 migrants per year are reported to have entered the Evros prefecture in Greece by crossing the 206 km long Greek-Turkish border. The border follows the Evros River, hence the name of this prefecture [2]. During 2010, the number of migrants increased more than tenfold to 47,000 in the same region [3]. In October 2010, the European Agency for the Management of Operational Cooperation at the External Borders of the Member States of the European Union (FRONTEX) , reported that Greece accounts for 90% of all detections of illegal border crossings in the European Union (EU) [4]. For the purpose of this report, 'migrants' are defined as including refugees, asylum seekers, displaced populations, irregular migrants and in some cases labour migrants, as defined by the International Organization for Migration (IOM) in the Glossary on Migration [1].

In the first months of 2011, the average number of migrants detected per day was about 58 in the Evros prefecture [5]. Migrants enter the region mainly by crossing the Evros. When water levels are high, migrants either swim or ferry over in small boats. In 2011, several persons have been reported to have died when crossing the border [6].

The increasing influx of migrants without documents in early 2011 led to massive overcrowding of the detention centres in the Evros prefecture and worsened the already poor humanitarian conditions that have been repeatedly criticised since 2005 by the European Committee for the Prevention of Torture and Inhuman or Degrading Treatment or Punishment and Amnesty International [7–11]. In addition to worsening humanitarian conditions, the overcrowding also increases the risk for the spread of communicable diseases such as tuberculosis, diarrhoea and respiratory infections [12].

The Greek Ministry of Health and Social Solidarity (MoHSS) had sought to address the health-related implications of overcrowding by setting up a project for the provision of healthcare inside the detention centres.

Additionally in March 2011, the Greek Health Minister invited representatives from the European Centre for Disease Prevention and Control (ECDC) and the World Health Organization (WHO) Regional Office for Europe to conduct a joint mission to the Thrace region.

The objective of the mission was to support Greek health authorities in assessing the public health situation in the detention centres for irregular migrants, with emphasis on communicable diseases, over-

all health condition of the migrants and potential additional needs.

Demographic features of migrants

The geographical and cultural origin of migrants seems to vary. In March 2011, FRONTEX reported the largest groups of migrants in Evros as coming from Afghanistan (24%), Pakistan (14%) and Bangladesh (12%) [5]. For the earlier period of August to December 2010, the Hellenic Centre for Disease Control and Prevention (HCDCP) had reported a total of 31 countries of origin, with Afghanistan (33%) and the Occupied Palestinian Territory (28%) being the most common, followed by Somalia (7%), Morocco (6%), Iraq and Algeria (4% each).

Data on age and sex were available for 1,229 migrants apprehended at the Evros river outposts between 8 August 2010 and 12 December 2010. Most of them (1,017; 83%) were male. Adolescents and young adults aged 12 to 40 years accounted for 91% (Data not shown).

Detention

Police authorities report that most migrants arrive without valid identification papers, such as passports or ID cards, and intend to claim asylum, mostly in other European countries such as Denmark, France, Germany, Norway, Sweden, Switzerland and the United Kingdom. According to Greek law, persons without identity papers shall be detained in closed centres for a maximum period of up to six months, until identification and nationality are validated. Lack of identity documents complicates the already bureaucratic and time-consuming process of seeking legal status [13], especially when the competent authorities are overburdened, and therefore prolongs the period of detention.

There are seven centres for migrants in the Eastern Macedonia and Thrace region, of which six are in the Evros prefecture [14]. One serves as a screening centre for entry assessment only and one for imprisoned traffickers only. Local police authorities are responsible for security and supplies in these centres which are often located within the local police stations. Most detention centres are regular police prisons having one to six cells, of variable size and with a maximum of two toilets and showers per cell. Only two centres have separate cells for women and families.

The five-month migrant healthcare project, 'Implementation of healthcare and psychosocial support activities for third country nationals that may require international protection in the area of Evros-Greece', was implemented in March 2011 [15]. It aimed at providing medical and psychosocial support to detained persons. It was funded by the EU (80%) and by the Greek national authorities (20%). Its implementation was under the responsibility and coordination of the HCDCP. Funding was limited to five months and ended in July 2011. Prior to March 2011, healthcare was mainly provided by medical doctors of the local authorities, non-governmental organisations such Médecins Sans Frontières (MSF) Greece and the HCDCP.

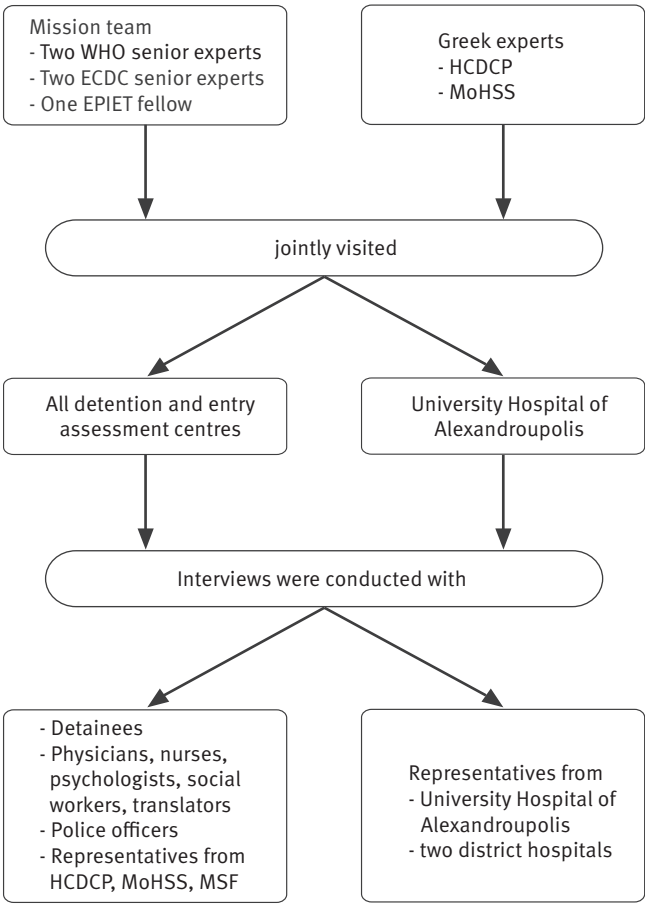
Assessment visit

In order to assess the public health situation of migrants in Greek detention centres, a joint mission was undertaken in April 2011 by the ECDC and the WHO Regional Office for Europe. The assessment was carried out through visits to detention centers and the University Hospital of Alexandroupolis, where interviews were carried out. The course of the visits and interviews in displayed in the Figure.

The mission team consisted of two senior experts from ECDC and WHO Regional Office for Europe and a fellow of the European Programme for Intervention Epidemiology Training (EPIET). The international team and Greek experts from the HCDCP and the MoHSS visited all detention and entry assessment centres, as well as the University Hospital of Alexandroupolis, where migrants are admitted if they need specialised

Figure

Course of visits and interviews, assessment visit to Greece, April 2011



ECDC:European Centre for Disease Prevention and Control; EPIET: European Programme for Intervention Epidemiology Training; HCDCP: Hellenic Centre for Disease Prevention and Control; MoHSS: The Greek Ministry of Health and Social Solidarity; MSF: Médecins Sans Frontières; WHO: World Health Organization

healthcare. The centre for traffickers was not visited. Unaccompanied minors (if stated age is less than 18 years) are sent to special centres for minors outside the Evros prefecture which minors can leave on demand. These centres were not visited during this mission.

In the detention centres, we conducted interviews with convenience samples of detained persons on their health, access to food, water, sleeping conditions, hygiene and what they regarded as the main problems of their situation. We also met representatives from MSF and gathered information on their assessment of the situation. Semi-structured interviews were conducted with representatives of the HCDCP, the MoHSS, the University Hospital of Alexandroupolis, two district hospitals, as well as with physicians, nurses, psychologists, social workers, translators and police officers at the detention and screening centres.

The 'health system crisis preparedness assessment method' [16], a tool available from the WHO, was used as framework during the three-day field visit. Minimum standards for occupancy (3.5m² per person) and hygiene conditions (one toilet per 20 persons) were assessed according to WHO emergency standards [17].

Visit findings

The visit findings are based on the mission teams' observations and semi-structured interviews. We interviewed two administrative employees of the migrant healthcare project and the programme manager, all from the HCDCP. In every centre, we interviewed (i) one to three police officers, (ii) one physician and one nurse working on site and (iii) ten to forty detainees. Of the staff that rotates between the centres, we interviewed one social worker, three translators and three psychologists.

Basic conditions

According to police authorities, a total of approximately 950 persons were detained in all five detention centres for migrants two days before our visit. Migrant fluctuation was high, as more than 200 persons had been released on the day prior to our visit, and more than 200 persons arrived in one of the centres during the two days of our visit. Occupancy varied between 75 in the smallest and 360 in the largest centre. According to the estimate of MSF representatives, the current number of detained persons therefore exceeded capacity two- to three-fold in four of the five detention centres (personal communication, MSF representatives, 6 April 2011). As far as the mission team could judge, none of the centres met the WHO minimum standard for occupancy of 3.5 m² per person.

We were able to obtain data on length of stay from 27 detainees. Among them, the median period of detention was 30 days (range five to 210 days). Police authorities were not able to give reliable estimations of proportions of minors because their number was fluctuating strongly. That opinion was shared by the longest serving on-site physician.

In the interviews with police authorities, the most pressing concern was the further deterioration of the problems due to overcrowding, as the numbers of migrants were expected to increase as soon as the river's water level dropped in summer.

It was confirmed by several sources that unlimited drinking water was available in sufficient quality and quantity apart from in one centre were detainees reported the tap water to have a brownish colour and bad taste. Police officers and the healthcare team confirmed that the tap water in the whole village has a brownish colour due to high iron rust levels. Food was provided by catering services on a daily basis and was assessed by healthcare workers and most detainees to be sufficient in both quantity and quality. Two or three meals a day were made available to the migrants, consisting of bread alone for breakfast, and bread with mixed salad for lunch and dinner. In one centre, detainees reported that rations were insufficient on days when many new migrants arrived in the centre. The visiting team noted that no centre had cooling facilities for the salad boxes.

The visiting team further noted that hygienic conditions of toilets, cleanliness of premises and availability of personal hygiene resources were sub-standard. Detainees reported a lack of soap and detergents as well as too few toilets and showers. In four of five detention centres, police and detainees confirmed that less than one toilet was available per 20 persons. In one centre, 79 persons had to share one toilet and one shower.

All of the centres were undersupplied with beds, mattresses and blankets. Beds/mattresses and blankets had to be shared by two to four persons in two centres, according to detainees. In the remaining three centres, a substantial number of detainees had to sleep on the concrete floor; it was observed by the visiting team and confirmed by detainees that thin industrial felts instead of mattresses were available for only the half of the detainees. Detainees had very limited (once every three to four days) or no access to an outside yard/physical exercise. This was confirmed by police present at the centres.

The physician of the largest centre reported an average of two fights per month during which detainees are injured and have to be isolated for their own protection. Ethnicity and religion were not considered for assigning detainees to cells. However, women or families were detained separately from men.

Health

According to the HCDCP, an entry assessment of all migrants, psychosocial and medical support upon request, as well as a telephone-based early warning

system were introduced under the newly established migrant healthcare project. Services were delivered by seven physicians, eight nurses, five psychologists and three social workers. The team was complemented by fourteen translators who covered Greek, English, French, Arabic, Farsi, Urdu, Pashto and Russian. Sustained healthcare provision to detained persons beyond the completion of the project is still under discussion. The services of the migrant healthcare project are described below.

Entry medical examination and assessment of all migrants

In all six visited screening and detention centres, healthcare for inmates was provided by one to two nurses and one physician as established by interviews with several parties.

After apprehension, all migrants undergo a health check that consists of questions about their medical history and a clinical examination. Detained migrants are additionally tested for tuberculosis (Mantoux test) and their blood samples screened for hepatitis B, Crimean Congo haemorrhagic fever and syphilis. According to detention centre healthcare staff and the HCDCP, migrants apprehended but released due to decisions by police authorities, undergo the entry examination but not blood or tuberculosis screening, as follow-up treatment is not possible.

Psychosocial support

Psychological and social support is offered to detainees by psychologists and social workers, assisted by cultural mediators who rotate between the centres. A particular focus is on supporting children and adolescents, especially unaccompanied ones. All new migrants are screened by psychologists with the assistance of translators. During the first session, a standardised questionnaire for each migrant is filled in. Once an environment of trust is created, more details are collected in further sessions. For migrants suffering traumatisation, psychologists provide counselling in single and group sessions and are responsible for psychiatric referrals. The type of session as well as the number and intervals of sessions are determined by psychologists case by case. Psychologists are supported by translators during the sessions when possible. Social workers support detainees in handling administrative matters such as identity confirmation.

Disease surveillance

Health conditions as diagnosed during entry assessment and follow-up examinations are systematically documented after release from detention with a delay of approximately one week for data entry. Diseases that need immediate and/or specialised care are reported by the medical staff in the detention centres via telephone to the healthcare project manager.

In addition to entry assessments, physicians and nurses conduct daily assessments of the health of detainees by visiting the cells and treating patients on request. They assume an outbreak if they find more than three persons in one cell with similar respiratory or gastroenteric symptoms or fever. Additionally, the project manager receives written summaries of the migrants' health from all detention centres on a daily basis.

The HCDCP and healthcare staff reported three cases of tuberculosis between March and mid-July 2011, but no outbreaks of communicable diseases were noted.

Vaccination

As part of the migrant healthcare project, children (‹18 years) are vaccinated against diphtheria, tetanus, pertussis, polio, measles, mumps and rubella. Adults are vaccinated against diphtheria, tetanus and polio. These vaccinations are administered to all detainees because the migrants without identity documents do not have vaccination cards and their vaccination status cannot be validated. The staff at detention centres is encouraged to get vaccinated against the same diseases as adult detainees. Vaccines against diphtheria, tetanus and polio are available for staff at the centres where they work.

Specialised healthcare

Migrants who need specialised healthcare are referred to the University Hospital of Alexandroupolis (670 beds) or one of two district hospitals (217 and 150 beds) in the Evros prefecture.

Conclusion

The main problem in all visited detention centres were the substandard hygiene conditions, especially overcrowding and lack of personal hygiene facilities, lack of basic supplies and lack of access to fresh air and physical exercise. The very poor humanitarian conditions in the centres needed to be improved urgently. In order to limit the risk for outbreaks of vaccine-preventable diseases, vaccination of detainees, healthcare workers and other staff in the centres should be continued. There was no evidence for immediate threats to the health of the Greek population originating from the migrants.

Considering the traumatisation many migrants have gone through, the psychosocial support services also need to be sustained and increased. The severe overcrowding should be addressed as it increases the risk for communicable diseases such as diphtheria, tetanus and polio spreading, psychosocial distress and the aggravation of traumatisation, as well as causing potentially violent conflicts.

As the migration route via the Evros region is increasingly used since 2009, and due to the unstable political situation in North Africa and the Middle East, an increased influx of migrants is to be expected with the falling water levels of the Evros River in summer, resulting in further deterioration of the already critical

situation in the Thrace region's detention centres. EU Member States should share public health best practices for managing detention centres.

According to the police authorities, the translators and detainees, the implementation of the migrant healthcare project has greatly improved access to healthcare including psychosocial support for migrants. The early warning system that was introduced by the migrant healthcare project, was based on personal communication, vulnerable to human and technical errors and depended on the presence of the project manager. During the time between the assessment visit and the submission of this article, a syndromic surveillance system was developed and successfully implemented within the framework of the EPIET programme [18].

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Full report

The ECDC Mission report [19] is available from http://www.ecdc.europa.eu/en/publications/Publications/1105_MIR_Joint_WHO_Greece.pdf

References

1. International Organization for Migration (IOM). Glossary on Migration [Internet]. Geneva: IOM; 2004 [accessed 2011 May 11]. Available from: http://www.iom.int/jahia/webdav/site/myjahiasite/shared/shared/mainsite/published_docs/serial_publications/Glossary_eng.pdf
2. Central Intelligence Agency (CIA) The world factbook, Greece. [accessed 2011 April 12]; Available from: https://www.cia.gov/library/publications/the-world-factbook/geos/gr.html
3. FRONTEX. Press Pack may 2011: FRONTEX; 2011 [accessed 2011 October 09]. Available from: http://www.frontex.europa.eu/newsroom/news_releases/art98.html
4. Frontex Frontex deploys Rapid Border Intervention Teams to Greece. Frontex European Union Agency; 2010 [accessed 2011 July 27]; Available from: http://www.frontex.europa.eu/newsroom/news_releases/art79.html.
5. FRONTEX RABIT Operation 2010 Ends, Replaced By JO Poseidon 2011. FRONTEX; 2011 [accessed 2011 May 11]; Available from: http://www.frontex.europa.eu/newsroom/news_releases/art98.html
6. Médecins Sans Frontières (MSF) [Internet]. Greece: Immediate action needed to improve unbearable living conditions.2011 [updated 2011 Jan 26; accessed 2011 May 11]; Available from: http://www.msf.org.uk/Evros_living_conditions_20110126.news
7. CPT. Report to the Government of Greece on the visit to Greece carried out by the European Committee for the Prevention of Torture and Inhuman or Degrading Treatment or Punishment (CPT) from 27 August to 9 September 2005 [Internet]. Strasbourg: CPT; 2006 [accessed 2011 May 11]. Available from: http://www.cpt.coe.int/documents/grc/2006-41-inf-eng.htm.
8. CPT. Report to the Government of Greece on the visit to Greece carried out by the European Committee for the Prevention of Torture and Inhuman or Degrading Treatment or Punishment (CPT) from 20 to 27 February 2007 [Internet]. Strasbourg: CPT; 2008 [accessed 2011 May 11]. Available from: http://www.cpt.coe.int/documents/grc/2008-03-inf-eng.htm.
9. CPT. Report to the Government of Greece on the visit to Greece carried out by the European Committee for the Prevention of Torture and Inhuman or Degrading Treatment or Punishment (CPT) from 17 to 29 September 2009 [Internet]. Strasbourg: CPT; 2010 [accessed 2011 May 11]. Available from: http://www.cpt.coe.int/documents/grc/2010-33-inf-eng.htm
10. CPT [Internet]. Strasbourg. Public statement concerning Greece. European Committee for the Prevention of Torture and Inhuman or Degrading Treatment or Punishment (CPT) 2011 [updated 2011 March 15; accessed 2011 May 11]; Available from: http://www.cpt.coe.int/documents/grc/2011-10-inf-eng.htm
11. Amnesty International [Internet]. Greece must urgently remedy deplorable detention conditions.2011 [updated 2011 March 16; accessed 2011 May 11]; Available from: http://www.amnesty.org/en/library/asset/EUR25/006/2011/en/14f92528-a129-42d4-9053-a25a99a2edba/eur250062011en.html
12. European Centre for Disease Prevention and Control (ECDC). Risk assessment: Situation in northern Africa/Libyan Arab Jamahiriya and the influx of migrants to Europe. 2011. 2011. Available from: http://ecdc.europa.eu/en/publications/Publications/Forms/ECDC_DispForm.aspx?ID=667
13. European Commission. Clandestino Project. Final Report. Athens: European Commission 2009.
14. GlobalDetentionProject Greece Detention Profile. GlobalDetentionProject; 2009 [updated November 2009; accessed 2011 May 11]; Available from: http://www.globaldetentionproject.org/countries/europe/greece/introduction.html
15. Hellenic Centre for Disease Prevention and Control (HCDCP) Evros Project. HCDCP; [accessed 2011 26 October]; Available from: http://www2.keelpno.gr/blog/?cat=10&lang=en&paged=2
16. World Health Organization (WHO). Health Needs Assessment, Malta [Internet]: WHO; 2011 [accessed 2011 July 01]. Available from: http://www.euro.who.int/__data/assets/pdf_file/0011/144011/Health_Needs_Assess_Malta_2011.pdf
17. World Health Organization (WHO). Communicable disease control in emergencies: a field manual [Internet]: WHO; 2005 [accessed 2011 May 11]. Available from: http://www.who.int/infectious-disease-news/IDdocs/whocds200527/ISBN_9241546166.pdf
18. Bosman A, Schimmer B, Coulombier D. Contribution of EPIET to public health workforce in the EU, 1995-2008. Euro Surveill. 2009;14(43).
19. European Centre for Disease Prevention and Control (ECDC). MISSION REPORT: Increased influx of migrants at the Greek–Turkish border: European Centre for Disease Prevention and Control (ECDC); 2011 [accessed 2011 May 28]. Available from: http://www.ecdc.europa.eu/en/publications/Publications/1105_MIR_Joint_WHO_Greece.pdf

Molecular epidemiology of human pathogens: how to translate breakthroughs into public health practice, Stockholm, November 2011

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This report outlines the main conclusions from an expert consultation on molecular epidemiology held on 22–23 November 2011, hosted and organised by the European Centre for Disease Prevention and Control (ECDC) [1]. The consultation brought together researchers, microbiologists and public health experts to discuss how public health can benefit from the recent scientific and technological advances in molecular microbiology, with special focus on the rapidly evolving next-generation sequencing technology.

Interplay of basic and public health microbiological investigations: case study of the *Escherichia coli* O104:H4 outbreak in Germany, 2011

Angelika Fruth (Robert Koch Institute, Germany), Flemming Scheutz (Statens Serum Institut, Denmark) and Martina Bielaszewska (University of Münster, Germany) described how laboratories at different levels contributed to the identification and detailed characterisation of the Shiga toxin-producing *E. coli* (STEC) O104:H4 strain during the 2011 outbreak in Germany [2-4]. The processes applied in the public health microbiology investigations underlined various challenges: obtaining relevant clinical isolates linked to outbreak cases at reference-laboratory level, coping with a high volume of tests and providing rapid feedback on antigenic and virulence profile information of the epidemic strain during the escalation phase of the outbreak.

For the first time in such a large epidemic of foodborne bacterial disease, the whole genome sequence of the pathogen was determined within days of recognition of the outbreak. The relative value of traditional microbiological methods in relation to whole genome sequencing (WGS) was discussed. The general opinion was that WGS approaches are rapidly evolving and will be a powerful tool in future public health emergencies, especially when the infectious agent is less well known. However, in this particular outbreak, traditional methods were effective for characterising the outbreak strain for epidemiological investigations. It was also considered that other factors related to laboratory investigations need to be strengthened. These include the requirement for stool cultures for pathogen isolation, access to strain collections and typing databases with laboratory information combined with epidemiological data, better communication channels between laboratories and epidemiological institutes and standardisation of traditional characterisation methods between human and food laboratories.

Scientific and technological advances in molecular microbiology: what's in it for public health?

Marc Achtman (Cork University, Ireland) challenged the existing definitions of a bacterial species and the ability of available laboratory methods to investigate population structures of different bacterial species. He argued for core genomic and gene polymorphism analyses, as opposed to serotyping and DNA fingerprinting methods, for the application of phylogenetic analyses to support epidemiological investigations [5]. No single approach can yet handle the wide range of population structures within the group of bacteria causing human disease, due to considerable heterogeneity in genomic diversity and clonality versus recombination between and within species. There are a number of promising developments especially regarding sequencing, but for applications in regional public health laboratories, scientific studies and technical solutions are still needed.

The use of sequencing information to investigate disease dynamics and phylogeographic mapping has a stronger tradition in virology. Anne-Mieke Vandamme (Rega Institute for Medical Research, Belgium) demonstrated how integration of sequencing, epidemiological and spatio-temporal data has been used to understand specific viral epidemics, such as the global migration events and selected national transmission

characteristics of HIV [6,7] and the rapid transmission of influenza A(H1N1) virus during the 2009 pandemic [8].

On behalf of Jacques Schrenzel (Geneva University Hospitals, Switzerland), Marc Struelens (ECDC) presented an overview of state-of-the-art instruments for genomic and proteomic analysis and discussed their possible future application in microbiology laboratories. The main message was that novel mass spectrometry technologies are being introduced into clinical microbiological laboratories for pathogen identification. An open question is whether these technologies will achieve the simplicity combined with higher resolution needed to provide subspecies strain profiling as well as primary diagnostic information in real time. Extrapolating current trends suggests that next-generation proteomic technologies will become routine in clinical laboratories and advanced sequencing applications in reference and research laboratory settings.

Typing as a component of epidemic preparedness and response support

A working group discussed experiences and identified challenges when adopting a validated typing method to operate a quality-assured system for collection and analysis of integrated epidemiological and microbiological data at the European level. Experiences of the European Legionnaires' Disease Surveillance Network (ELDSNET) in setting up the successful international system for sequence-based genotyping of *Legionella* were shared.

A network of engaged laboratories spanning all participating countries was considered key for utilising existing structures and expertise, defining disease-specific objectives, sampling frames and typing techniques, as well as collecting representative isolates.

Human pathogens encompass diverse organisms, but typing techniques should aim at maximum across-species flexibility. Important health events related to these organisms range in scale (endemic, epidemic and emerging) and therefore require different sentinel thresholds. Independent of disease-specific differences, key steps for developing effective typing networks were proposed. First, every pathogen-specific network has to agree on a minimum set of metadata (place, person, time, etc.). Second, a typing approach should be agreed upon, which would preferably be highly versatile, and generate data with suitable portability, reproducibility, timeliness, traceability and biological robustness. Third, a set of rules stipulating a suitable sampling frame should be agreed upon. Fourth, a web-based typing platform should be set up. The manner of submission, access and ownership of contributors must be similar to those of other repositories, e.g. the National Center for Biotechnology Information (NCBI).

The need for regularly conducted structured molecular typing surveys to calibrate the baseline population structure and molecular diversity of the pathogen of interest was identified. Such surveys can also explore the role of potential sources and reservoirs of human pathogens using flexible sampling frames.

A clear threat to sustainability is the unpredictability of funding in the field of European public health. It is therefore important to safeguard against loss of data and isolates by providing the necessary redundancy in terms of biobanking and mirrored databases. Effective collection of enhanced surveillance data is also dependent on centralised capacity-building initiatives, such as regular proficiency testing, training and certification.

Infections pose a potential threat to all European Union (EU) citizens and can only be tackled by joint interventions. Notwithstanding ECDC's clear mandate, EU Member States should provide the necessary support at national level, i.e. maintaining their countries' diagnostic and reference typing services. The need for advanced training and capacity building was also recognised, especially in the field of bioinformatics, and for developing cross-disciplinary competences of public health professionals, as provided by programmes such as the European Programme for Public Health Microbiology (EUPHEM).

Translational research for informing public health action

A second working group appraised if reliable predictions of communicable disease pattern and impact can be made by combining genomic and biological information with epidemiological and clinical data. Work at the Dutch National Institute for Public Health and the Environment (RIVM) was presented, describing efforts to identify risk factors and geographical origin for hepatitis A infection in the Netherlands via continuing collection of partial genome sequence information by a national network of clinical virology laboratories.

Although a large amount of data on pathogenicity has been published in the last 20 years, it was considered that a molecular microbiological risk assessment to foresee disease impact based on pathogen genotyping is not possible at this stage of scientific knowledge. However, data from major epidemiological studies of pathogens such as HIV, influenza virus, enterohaemorrhagic *E. coli* and *Staphylococcus aureus* indicate that genotypic markers can help predict the probability of disease progression or response to therapy. Several participants considered that genome sequence-based disease predictors were better characterised in virology than in bacteriology. It was agreed that much scientific work remains and EU-wide translational research studies should be initiated to increase the accuracy of these predictions. Access to comparative whole genome studies of large samples of bacterial pathogens linked to clinical and epidemiological data

would facilitate the detection of relevant epidemiological–biological associations.

Free sharing of genomic data was advocated, but existing difficulties were also recognised, for example, ownership, confidentiality, national policies, etc. To stimulate scientific progress, future databases hosting typing and epidemiological information combined with innovative bioinformatic solutions should have adjustable access levels, depending on the users and purposes of using the information. Quality assurance and agreed nomenclature for sequence-based typing need to be addressed in parallel to capacity-building initiatives, also in bioinformatics.

Next generation technologies, such as WGS, represent a window of opportunity for EU-wide typing for surveillance in the coming years. Intersectoral collaborations in research networks, combining human and veterinary bacteriology, virology and epidemiology with genomic bioinformatics, molecular biology and population genetics should be facilitated. Guidance and facilitation by ECDC to achieve the goals of common nomenclature as well as intersectoral collaboration and capacity building would be helpful.

Optimising use of novel typing technology for public health

A third working group considered whether novel proteomic and genomic technologies will replace existing methodologies in public health microbiological laboratories within five years. Data were presented showing that WGS applications are already used in the majority of large outbreaks in Europe and should gradually replace currently used microbiological characterisation methodologies or improve them by identification of novel targets.

The discussion was focused on genomic methods: gaps and challenges in routine application of WGS in epidemiological typing were examined. There was no consensus about whether WGS will replace current typing techniques. Arguments against the use of WGS were that currently too much work is required for a small amount of additional useful data, the relative expense and that deriving phenotype from genotype remains an imperfect science.

A number of key hurdles for implementation of WGS for public health purposes were identified. Without either well-utilised local instrumentation or contact with larger sequencing institutes, many public health institutes are excluded from the possibility of using WGS routinely. The time from sampling to results is generally too long to use WGS for daily surveillance. A major unresolved question was how genome data should be analysed for epidemiological characterisation. Existing analysis platforms are still wanting in terms of user friendliness and the inability to produce complete and closed genomes risks fragmenting WGS techniques and analysis methods. Harmonisation of quality parameters for WGS data production is needed. Routine removal of genetic regions with high variation gave rise to concerns that too much information is left out of analyses – a concern for the analysis of mobile genetic elements that are of particular interest for public health.

To reduce WGS data to epidemiologically useful information is far from trivial. As it was found desirable to work with WGS data in a way similar to a conventional nomenclature of genotypes, several possibilities were examined, including an extended multi-locus sequence typing (MLST)-type classification, single nucleotide polymorphism-based classification or genome grouping into broader 'natural' lineages. These possible approaches to derive a 'type' are all hampered by considerable feasibility problems and unresolved ambiguities. The issue was thus left open, with the consideration that it was perhaps time to abandon the concept of 'type'.

Some other unmet needs for public health application of WGS involved the central storage and curation of data. ECDC along with other experts could be involved in setting the design and accreditation standards for genome-based surveillance databases. ECDC could take part in creating a system of unique identifiers and link these to the European Surveillance System (TESSy). In addition, ECDC could support disease-specific studies on the effectiveness of WGS analysis for public health applications such as epidemic investigations.

Updates of ECDC and EU projects related to molecular surveillance and use of new laboratory methods

Harry Vennema (RIVM, the Netherlands) presented the European Commission-funded Global exchange of viral sequences to underpin response to health threats (GESTURE) project [9]. The principles agreed in this project are planned to be discussed for possible implementation in the World Health Organization's upcoming World Health Assembly. Dag Harmsen (University of Münster, Germany) gave examples of how WGS methodologies have been applied in three outbreak investigations: STEC in Germany (2011) [10], *Klebsiella* OXA-48 in the Netherlands (2011) and *Neisseria meningitidis* in Germany (2001–2006). In two of these, WGS was performed in real time in parallel to traditional investigations, illustrating how sequencing technology can become a method of choice in these situations. Finally, Ivo Van Walle (ECDC) presented the ECDC plan to set up a generic platform for handling microbial typing data. The system, an extension of TESSy, will be piloted for a limited number of pathogens and typing methods and is planned to be released in spring 2012.

Conclusions

The conference clearly illustrated that recent advances in molecular microbial characterisation, primarily by WGS analysis, are opening up tremendous new scientific opportunities for a better understanding of

the pathogenicity, evolution and spread of human pathogens and the epidemiology of the diseases they cause. This technology has considerable potential for improving the resolution and predictive value of microbial typing as applied to public health objectives such as disease surveillance and epidemic investigation. However, many operational hurdles need to be addressed in the coming years, to define the methods to produce, analyse, share genomic information combined with epidemiological data in a meaningful way and build the capacity and fit-for-purpose data management platforms for public health microbiology. ECDC will follow up on the recommendations of the consultation to play the role of a facilitator, bridging the scientific and public health communities to jointly address the generic and disease-specific challenges identified.

References

1. European Centre for Disease Prevention and Control (ECDC). Expert consultation: Breakthroughs in molecular epidemiology of human pathogens - how to translate into public health practice. Stockholm: ECDC. [Accessed 12 Jan 2012]. Available from: http://www.ecdc.europa.eu/en/press/events/Lists/Events/ECDC_DispForm.aspx?List=43564830%2D6b8a%2D442f%2D84e7%2D2495fa49489b&ID=184&Source=http%3A%2F%2Fstaging%2Eecdcdmz%2Eeuropa%2Eeu%2Fen%2Fpress%2Fevents%2FLists%2FEvents%2FAllItems%2Easpx%3FSortField%3DCreated%26SortDir%3DDesc%26View%3D%257bB9767274%252d1B4F%252d4663%252dACD3%252d0F569564D9A8%257d
2. Scheutz F, Nielsen EM, Frimodt-Møller J, Boisen N, Morabito S, Tozzoli R, et al. Characteristics of the enteroaggregative Shiga toxin/verotoxin-producing Escherichia coli O104:H4 strain causing the outbreak of haemolytic uraemic syndrome in Germany, May to June 2011. Euro Surveill. 2011;16(24):pii=19889. Available from: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19889
3. Bielaszewska M, Mellmann A, Zhang W, Köck R, Fruth A, Bauwens A, et al Characterisation of the Escherichia coli strain associated with an outbreak of haemolytic uraemic syndrome in Germany, 2011: a microbiological study. Lancet Infect Dis. 2011;11(9):671-6.
4. Rasko DA, Webster DR, Sahl JW, Bashir A, Boisen N, Scheutz F, et al. Origins of the E. coli strain causing an outbreak of hemolytic-uremic syndrome in Germany. N Engl J Med. 2011;365(8):709-17.
5. Didelot X, Bowden R, Street T, Golubchik T, Spencer C, McVean G, et al. Recombination and population structure in Salmonella enterica. PLoS Genet. 2011;7(7):e1002191.
6. Paraskevis D, Pybus O, Magiorkinis G, Hatzakis A, Wensing AM, van de Vijver DA, et al. Tracing the HIV-1 subtype B mobility in Europe: a phylogeographic approach. Retrovirology. 2009;6:49.
7. Faria NR, Suchard MA, Abecasis A, Sousa JD, Ndembi N, Bonfim I, et al. Phylodynamics of the HIV-1 CRF02_AG clade in Cameroon. Infect Genet Evol. 2011 May 4. [Epub ahead of print].
8. Lemey P, Suchard M, Rambaut A . Reconstructing the initial global spread of a human influenza pandemic: a Bayesian spatial-temporal model for the global spread of H1N1pdm. PLoS Curr. 2009; 1:RRN1031.
9. Executive Agency for Health and Consumers (EAHC). Global exchange of viral sequences to underpin response to health threats (GESTURE). Project No 20084153. EAHC: Luxembourg. [Accessed 12 Jan 2012]. Available from: http://ec.europa.eu/eahc/projects/database.html?prjno=20084153
10. Mellmann A, Harmsen D, Cummings CA, Zentz EB, Leopold SR, Rico A, et al. Prospective genomic characterization of the German enterohemorrhagic Escherichia coli O104:H4 outbreak by rapid next generation sequencing technology. PLoS One. 2011;6(7):e22751.