Impact factor 4.659

Eurosurveillance

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

Vol. 19 | Weekly issue 31 | 07 August 2014

Rapid communications

Surveillance and outbreak reports

News

ecdc EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

www.eurosurveillance.org

West Nile virus lineage 2 isolated from *Culex modestus* mosquitoes in the Czech Republic, 2013: expansion of the European WNV endemic area to the North?

I Rudolf[1], T Bakonyi[2,3], O Šebesta[1], J Mendel[1], J Peško[1], L Betášová[1], H Blažejová[1], K Venclíková[1,4], P Straková[1,4], N Nowotny[2,5], Z Hubálek (zhubalek@brno.cas.cz)[1]

1. Institute of Vertebrate Biology, Academy of Sciences, v.v.i., Brno, Czech Republic
2. Viral Zoonoses, Emerging and Vector-Borne Infections Group, Institute of Virology, University of Veterinary Medicine, Vienna, Austria
3. Department of Microbiology and Infectious Diseases, Faculty of Veterinary Science, Szent István University, Budapest, Hungary
4. Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic
5. Department of Microbiology and Immunology, College of Medicine and Health Sciences, Sultan Qaboos University, Muscat, Oman

Citation style for this article:
Rudolf I, Bakonyi T, Šebesta O, Mendel J, Peško J, Betášová L, Blažejová H, Venclíková K, Straková P, Nowotny N, Hubálek Z. West Nile virus lineage 2 isolated from Culex modestus mosquitoes in the Czech Republic, 2013: expansion of the European WNV endemic area to the North?. Euro Surveill. 2014;19(31):pii=20867. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20867

Article submitted on 24 July 2014 / published on 07 August 2014

We report the detection and isolation of four almost identical strains of West Nile virus (WNV) lineage 2 from *Culex modestus* mosquitoes collected at three fish ponds in South Moravia, Czech Republic, during August 2013. Phylogenetic analysis demonstrated that the Czech WNV strains isolated are closely related to Austrian, Italian and Serbian strains reported in 2008, 2011 and 2012, respectively. Our findings show the current northernmost range of lineage 2 WNV in Europe.

In South Moravia in the Czech Republic, surveillance activities for mosquitoes and mosquito-borne pathogens have been carried out for several decades, but until our findings in 2013 presented here, WNV lineage 2 (WNV-2) had not been detected.

Background

WNV is a mosquito-borne virus (genus *Flavivirus*; family *Flaviviridae*) that is widely distributed in Africa, the Middle East, Asia and southern Europe [1] and was recently introduced in the Americas [2]. WNV circulates in natural foci between birds (as amplifying hosts) and bird-feeding mosquitoes, in Europe principally *Culex pipiens* and *Cx. modestus* [3]. Humans and horses are considered accidental dead-end hosts. Most individuals infected with WNV are asymptomatic. Symptoms may develop in 20–40% of people with WNV infection, most frequently characterised as influenza-like symptoms, (West Nile fever (WNF)). Less than 1% of infected individuals develop severe neuroinvasive disease, which can be classified into three main clinical syndromes: West Nile meningitis, West Nile encephalitis and acute flaccid paralysis [4].

Several human and/or equine WNF outbreaks have occurred in the last decades in Europe, for example, in Romania (1996), Italy (1998) and Russia (1999) [1]. From 2008 onwards, an unexpected explosive spread of WNV-2, which resulted in several hundreds of human neuroinvasive cases, has been documented in Hungary, Greece and Serbia [5-7].

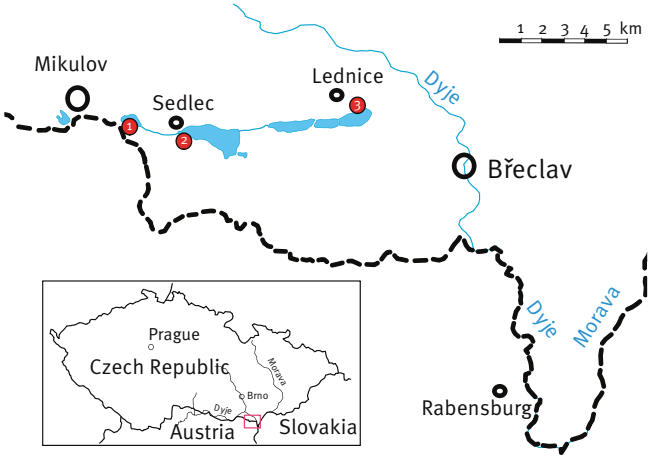
In the Czech Republic, three identical strains of WNV (proposed genomic lineage 3: Rabensburg) were isolated from *Cx. pipiens* and *Aedes rossicus* mosquitoes in 1997, 1999 and 2006 [8,9]. Although neutralising antibodies against WNV have been found rarely in humans in the Czech Republic, two confirmed cases of WNF in humans were reported after heavy floods in 1997 [10]. In addition, WNV-specific antibodies have been detected in resident wild bird species [11]. The above rare traces of WNV infections in the Czech Republic before 2008 were most likely due to WNV lineage 1. Sera collected from 163 horses, originating from 43 out of 77 administrative districts of the Czech Republic between 2008 and 2011, all proved negative for WNV antibodies [12]. Because of the rapidly changing epidemiological situation regarding WNF in Europe, we decided to perform virological surveillance of mosquitoes for WNV and related pathogenic flaviviruses (e.g. Usutu virus) to investigate the epidemiological relevance of WNF in the Czech Republic.

Study site

In this study, mosquitoes were collected within reed belts (*Phragmition communis* alliance) of the fish ponds 'Nesyt' (48 ° 46'35''N, 16 ° 42'05''E; 176 m above sea level (a.s.l.)) and 'Nový' (48 ° 46'57''N, 16 ° 40'13''E; 177 m a.s.l.) at Mikulov, and the fish pond 'Mlýnský' at Lednice (48 ° 47'19''N, 16 ° 49'2''E; 175 m a.s.l.) during July and August 2013 (Figure 1). The climate at the ponds is relatively warm and dry: the mean annual air temperature is 9.1 °C (January –1.8 °C, July 19.2 °C); the mean annual precipitation is 571 mm

Figure 1

Locations of three study sites for *Culex modestus* trapping, South Moravia, Czech Republic, July–August 2013



Fish ponds:
1 Nový
2 Nesyt
3 Mlýnský

(range: 284–919 mm) (data purchased from the Czech Hydrometeorological Institute). A total of 30 species of birds have been recorded breeding in the reed belts; 51 other avian species breed in the close surroundings of the ponds and an additional 54 wild wetland and terrestrial bird species visit this habitat during their seasonal movements. Mosquitoes in South Moravia comprise 30 species of the genera *Anopheles, Aedes, Ochlerotatus, Culex, Culiseta, Coquillettidia* and *Uranotaenia* [13].

Mosquito collection, molecular screening and virus isolation attempts

Mosquitoes were captured using CDC minilight-CO_2-baited traps (EVS CO_2 Mosquito Trap, BioQuip Products, Inc., United States) placed at a height of approximately 1 m above the ground. The traps were run on two successive nights at two-week intervals. The caught insects were transported to the laboratory of the Institute of Vertebrate Biology, Brno, Czech Republic, in cooled flasks (4 to 8 °C) and stored at −65 °C until examination. They were identified under a stereomicroscope and monospecific pools consisting of 50 *Cx. modestus* females were homogenised in 1.5 ml cooled phosphate buffered saline pH 7.4 supplemented with 0.4% bovine serum albumin (Sigma) and antibiotics (PBS-BSA) and centrifuged.

Viral RNA was extracted from 140 µl mosquito homogenates using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany). Oligonucleotide primers targeting the NS5 region of flaviviruses were used for screening [14]. If samples were positive, a set of WNV-specific primers were used in continuous reverse transcription (RT)-PCRs for amplification of overlapping genome fragments that covered the entire genome sequences of the detected viruses [15]. Amplification products were sequenced directly (Microsynth, Balgach, Switzerland), sequences were aligned and compiled, and identified by basic local alignment search tool (BLAST) search against the GenBank database. The WNV sequences were aligned with 25 complete or nearly complete lineage 2 WNV sequences deposited in GenBank database. Phylogenetic and molecular evolutionary analyses were conducted using neighbor-joining and maximum likelihood algorithms (MEGA version 6 [16], with 1,000 replicates for bootstrap testing) and inferred genetic relationships were shown in a phylogram.

Mosquito homogenates of WNV PCR-positive samples (20 µl) were inoculated intracerebrally into specified pathogen-free suckling ICR mice (SM). The brains of SM that succumbed to the infection were homogenised in PBS-BSA, centrifuged and passaged (intracerebrally) in a new batch of SM. Bacterial sterility of the suspensions was checked in meat-peptone and thioglycollate broths incubated at 37 °C [9].

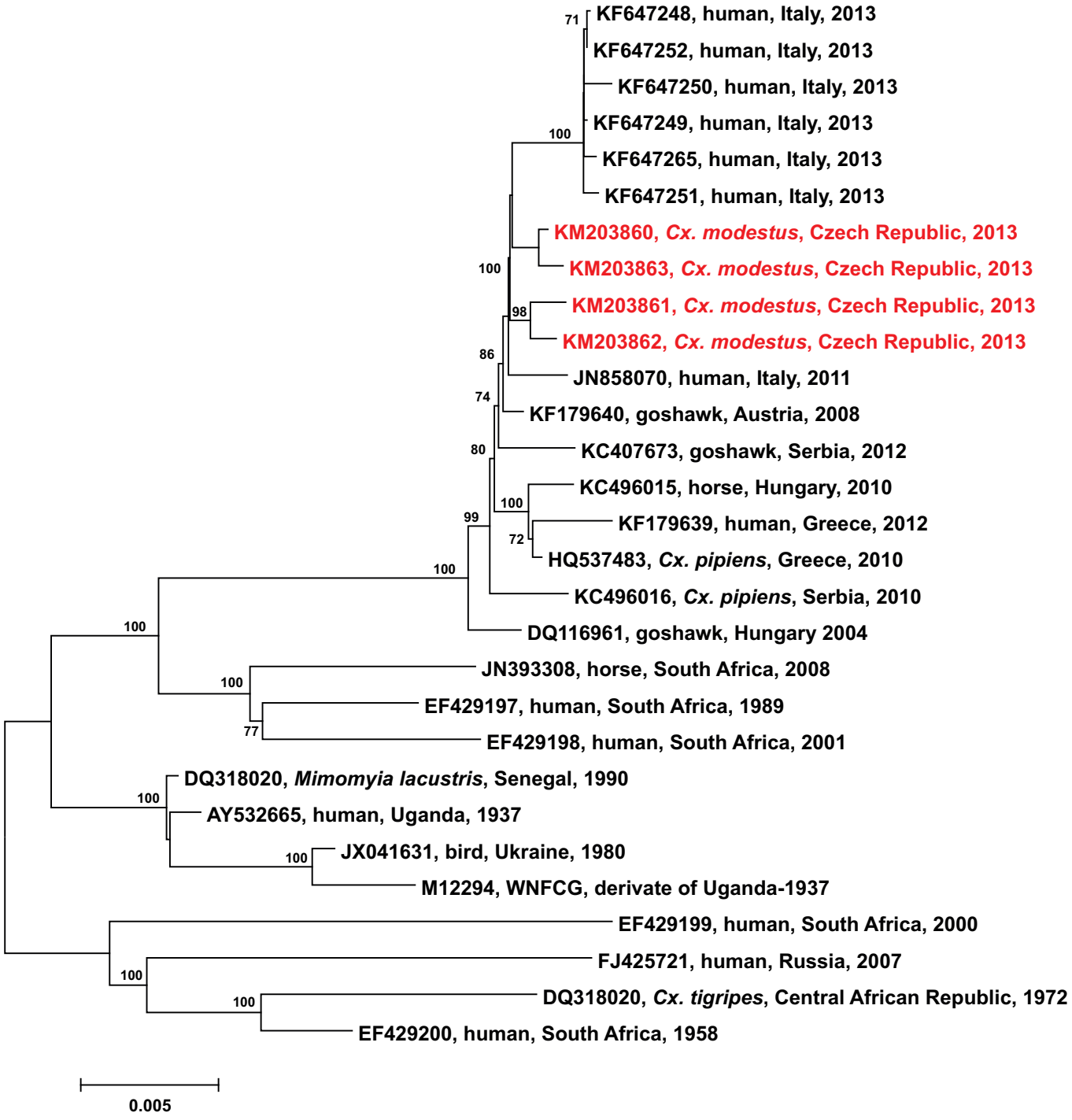
West Nile virus prevalence in *Culex modestus* mosquitoes

A total of 32,500 female *Cx. modestus* mosquitoes in 650 pools were examined for flaviviruses by RT-PCR. RNA of lineage 2 WNV was detected in four pools of insects collected in August 2013: number 13-104 (collected at Nový fish pond), number 13-329 (collected at Nesyt fish pond), number 13-479 (collected at Mlýnský fish pond) and number 13-502 (collected at Mlýnský fish pond). The minimum prevalence rate of WNV in the examined mosquito pools was therefore 1:8,125 (0.012%). All WNV-2-positive mosquito homogenates were inoculated into SM. While number 13-329 did not kill any mice, the three others did: number 13-104 killed 6 of 11 inoculated SM within 7–8 days post inoculation (DPI) and the average survival time (AST) of SM was 7.7 days; number 13-479 killed 8 of 9 inoculated SM (6–7 DPI; AST 6.1 days); and number 13-502 killed 7 of 10 SM (6–8 DPI; AST 6.4 days). Interestingly, experimentally non-infected mothers of mice inoculated with homogenates from all three infective pools succumbed to infection seven to eight days after cannibalising their dead SM, and WNV was demonstrated by real-time RT-PCR in high concentration (10^7 RNA copies/ml) in the mothers' brains but not in their livers or spleens. This finding supports the hypothesis of oral infection as a (rare) alternative route of WNV transmission, for example, in raptors.

Phylogenetic analysis based on complete WNV-2 genome sequences demonstrated that the four Czech WNV strains identified form two closely related groups: number 13-104 (GenBank: KM203860) with number 13-502 (GenBank: KM203863) and number 13-329 (GenBank: KM203861) with number 13-479 (GenBank: KM203862) and that they cluster together with WNV strains from an Austrian goshawk (isolated in 2008; GenBank: KF179640), Serbian *Cx. pipiens* (in 2012; GenBank: KC407673) and Italian human (in 2011; GenBank: JN858070), while they differ partially from

Figure 2

Phylogenetic positioning of four West Nile virus strains identified in *Culex modestus* mosquitoes, South Moravia, Czech Republic, August 2013



WNV: West Nile virus.

The complete genome nucleotide sequences of the four WNV strains from the Czech Republic (marked in red) were analysed together with representative lineage 2 WNV strains by the neighbor-joining method. GenBank accession numbers, isolation sources, countries of origins and isolation years are indicated at the branches. Supporting (>70%) bootstrap values of 1,000 replicates are displayed at the nodes. The horizontal bar shows genetic distance.

other European WNV-2 strains compared. However, they are all in the same clade (i.e. central and south European WNV-2), while WNV-2 strains from Africa and Russia form distinct clades (Figure 2). Maximum likelihood analysis resulted in a similar tree topology. Although three of the four Czech isolates were found to be neuropathogenic in SM, these virus strains do not carry the putative virulence marker P249 within the NS3 region [17,18].

Conclusions

The discovery of WNV-2 in the Czech Republic has added another country to the list of WNV risk areas in Europe. It also shows that two different lineages of WNV (lineages 2 and 3) co-circulate in the country and that *Cx. modestus* mosquito is a potential vector of WNV in reed belts of South Moravian fish ponds. This ornithophilic mosquito might play an important role in the bird–mosquito cycle of WNV in central Europe.

Our study highlights the need for epidemiological surveillance of (re-)emerging mosquito-borne viruses in central Europe. The seasonal peak activity of the adult *Cx. modestus* population in central Europe is from the beginning of July to late September [19]. Usually, the females do not enter buildings, but readily bite humans outdoors often during the day, at sun- and wind-exposed places, causing a nuisance, especially in late summer when floodwater *Aedes* and *Ochlerotatus* mosquito species have already vanished [19]. The isolation of neuroinvasive WNV strains in South Moravian fish ponds (in a popular recreational and camping area during the summer) raises the question of a possible risk of a local WNF outbreak. Given the mild climate of the 2013–14 winter, we can only speculate on the possible emergence of WNF in this year's WNV season, if favourable conditions for mass breeding of mosquitoes occur. To date, no human WNF cases have been recorded this season, which has just begun (the WNV season in central Europe starts mid-July and the majority of cases are seen in September). While infectious disease specialists in the region are aware of the WNV situation, local general practitioners should also be aware of the circulation of WNV in this area and take it into account during differential diagnosis of late-summer neuroinfections.

Acknowledgements

This study was funded by the European Union grant FP7-261504 EDENext. The publication is catalogued by the EDENext Steering Committee as EDENext244.

Conflict of interest

None declared.

Authors' contributions

IR, ZH: designed, coordinated and supervised the study, performed laboratory testing, and wrote the manuscript; TB, JM: carried out sequence analysis, processed phylogenetic data, read and revised the manuscript; LB, HB, JP, PS, KV: trapped the mosquitoes, performed molecular analyses, read and revised the manuscript; OS: trapped the mosquitoes and performed their identification, read and revised the manuscript; NN: analysed data, wrote and revised the manuscript.

References

1. Hubálek Z, Halouzka J. West Nile fever - a reemerging mosquito-borne viral disease in Europe. Emerg Infect Dis. 1999;5(5):643-50. http://dx.doi.org/10.3201/eid0505.990506
2. Jia XY, Briese T, Jordan I, Rambaut A, Chi HC, Mackenzie JS, et al. Genetic analysis of West Nile New York 1999 encephalitis virus. Lancet. 1999;354(9194):1971-2. http://dx.doi.org/10.1016/S0140-6736(99)05384-2
3. Hubálek Z. European experience with the West Nile virus ecology and epidemiology: could it be relevant for the New World? Viral Immunol. 2000;13(4):415-26. http://dx.doi.org/10.1089/vim.2000.13.415
4. Kramer LD, Li J, Shi PY. West Nile virus. Lancet Neurol. 2007;6(2):171-81. http://dx.doi.org/10.1016/S1474-4422(07)70030-3
5. Bakonyi T, Ferenczi E, Erdélyi K, Kutasi O, Csörgő T, Seidel B, et al. Explosive spread of a neuroinvasive lineage 2 West Nile virus in Central Europe, 2008/2009. Vet Microbiol. 2013;165(1-2):61-70. http://dx.doi.org/10.1016/j.vetmic.2013.03.005
6. Pervanidou D, Detsis M, Danis K, Mellou K, Papanikolaou E, Terzaki I, et al. West Nile virus outbreak in humans, Greece, 2012: third consecutive year of local transmission. Euro Surveill. 2014;19(13):pii=20758.
7. Popović N, Milošević B, Urošević A, Poluga J, Lavadinović L, Nedelijković J, et al. Outbreak of West Nile virus infection among humans in Serbia, August to October 2012. Euro Surveill. 2013;18(43):pii=20613.
8. Bakonyi T, Hubálek Z, Rudolf I, Nowotny N. Novel flavivirus or new lineage of West Nile virus, Central Europe. Emerg Infect Dis. 2005;11(2):225-31. http://dx.doi.org/10.3201/eid1102.041028
9. Hubálek Z, Rudolf I, Bakonyi T, Kazdová K, Halouzka J, Šebesta O, et al. Mosquito (Diptera: Culicidae) surveillance for arboviruses in an area endemic for West Nile (lineage Rabensburg) and Tahyna viruses in Central Europe. J Med Entomol. 2010;47(3):466-72. http://dx.doi.org/10.1603/ME09219
10. Hubálek Z, Savage HM, Halouzka J, Juřicová Z, Sanogo YO, Lusk S. West Nile virus investigations in South Moravia, Czechland. Viral Immunol. 2000;13(4):427-33. http://dx.doi.org/10.1089/vim.2000.13.427
11. Hubálek Z, Halouzka J, Juřicová Z, Šikutová S, Rudolf I, Honza M, et al. Serologic survey of birds for West Nile flavivirus in southern Moravia (Czech Republic). Vector Borne Zoonotic Dis. 2008;8(5):659-66. http://dx.doi.org/10.1089/vbz.2007.0283
12. Hubálek Z, Ludvíková E, Jahn P, Treml F, Rudolf I, Svobodová P, et al. West Nile virus equine serosurvey in the Czech and Slovak Republics. Vector Borne Zoonotic Dis. 2013;13(10):733-8. http://dx.doi.org/10.1089/vbz.2012.1159
13. Šebesta O, Gelbič I, Minář J. Mosquitoes (Diptera: Culicidae) of the Lower Dyje River Basin (Podyjí) at the Czech–Austrian border. Cent Eur J Biol. 2012;7(2):288-98. http://dx.doi.org/10.2478/s11535-012-0013-8
14. Scaramozzino N, Crance JM, Jouan A, DeBriel DA, Stoll F, Garin D. Comparison of flavivirus universal primer pairs and development of a rapid, highly sensitive heminested reverse transcription-PCR assay for detection of flaviviruses targeted to a conserved region of the NS5 gene sequences. J Clin Microbiol. 2001;39(5):1922-7. http://dx.doi.org/10.1128/JCM.39.5.1922-1927.2001
15. Bakonyi T, Ivanics E, Erdélyi K, Ursu K, Ferenczi E, Weissenböck H, et al. Lineage 1 and 2 strains of encephalitic West Nile virus, central Europe. Emerg Infect Dis. 2006;12(4):618-23. http://dx.doi.org/10.3201/eid1204.051379
16. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol. 2013;30(12):2725-9. http://dx.doi.org/10.1093/molbev/mst197
17. Brault AC, Huang CY, Langevin SA, Kinney RM, Bowen RA, Ramey WN, et al. A single positively selected West Nile viral mutation confers increased virogenesis in American crows. Nat Genet. 2007;39(9):1162-6. http://dx.doi.org/10.1038/ng2097
18. Papa A, Bakonyi T, Xanthopoulou K, Vázquez A, Tenorio A, Nowotny N. Genetic characterization of West Nile virus lineage 2, Greece, 2010. Emerg Infect Dis. 2011; 17(5):920-2. http://dx.doi.org/10.3201/eid1705.101759
19. Becker N, Petrič D, Zgomba M, Boase C, Madon M, Dahl C, et al. Mosquitoes and their control, 2nd ed. Heidelberg: Springer; 2010. http://dx.doi.org/10.1007/978-3-540-92874-4

A multi-country outbreak of *Salmonella* Newport gastroenteritis in Europe associated with watermelon from Brazil, confirmed by whole genome sequencing: October 2011 to January 2012

L Byrne (lisa.byrne@phe.gov.uk)[1], I Fisher[1], T Peters[2], A Mather[3], N Thomson[3], B Rosner[4], H Bernard[4], P McKeown[5], M Cormican[6], J Cowden[7], V Aiyedun[1], C Lane[1], on behalf of the International Outbreak Control Team[8]

1. Gastrointestinal, Emerging and Zoonotic Infections, Centre for Infectious Disease Surveillance and Control, Public Health England, Colindale, London, United Kingdom
2. Gastrointestinal Bacteria Reference Unit, Centre for Infectious Disease Surveillance and Control, Public Health England, Colindale, London, United Kingdom
3. Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, United Kingdom
4. Robert Koch Institute, Department for Infectious Disease Epidemiology, Berlin, Germany
5. Health Protection Surveillance Centre, Dublin, Ireland
6. National *Salmonella*, *Shigella*, *Listeria* Reference Laboratory, Galway University Hospital, Newcastle Road, Galway, Ireland
7. Health Protection Scotland, Glasgow, United Kingdom
8. The members of the team are listed at the end of the article.

Citation style for this article:
Byrne L, Fisher I, Peters T, Mather A, Thomson N, Rosner B, Bernard H, McKeown P, Cormican M, Cowden J, Aiyedun V, Lane C, on behalf of the International Outbreak Control Team. A multi-country outbreak of *Salmonella* Newport gastroenteritis in Europe associated with watermelon from Brazil, confirmed by whole genome sequencing: October 2011 to January 2012. Euro Surveill. 2014;19(31):pii=20866. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20866

Article submitted on 10 April 2013 / published on 07 August 2014

In November 2011, the presence of *Salmonella* Newport in a ready-to-eat watermelon slice was confirmed as part of a local food survey in England. In late December 2011, cases of *S.* Newport were reported in England, Wales, Northern Ireland, Scotland, Ireland and Germany. During the outbreak, 63 confirmed cases of *S.* Newport were reported across all six countries with isolates indistinguishable by pulsed-field gel electrophoresis from the watermelon isolate. A subset of outbreak isolates were whole-genome sequenced and were identical to, or one single nucleotide polymorphism different from the watermelon isolate. In total, 46 confirmed cases were interviewed of which 27 reported watermelon consumption. Further investigations confirmed the outbreak was linked to the consumption of watermelon imported from Brazil. Although numerous *Salmonella* outbreaks associated with melons have been reported in the United States and elsewhere, this is the first of its kind in Europe. Expansion of the melon import market from Brazil represents a potential threat for future outbreaks. Whole genome sequencing is rapidly becoming more accessible and can provide a compelling level of evidence of linkage between human cases and sources of infection, to support public health interventions in global food markets.

Introduction

On 28 November 2011, as part of a local food survey, Public Health England (PHE; formerly the Health Protection Agency) Food Water and Environment (FWE) Laboratory in Preston, England, confirmed the presence of *Salmonella* in a ready to eat watermelon slice purchased from a major supermarket retailer. The isolate was sent to the Gastrointestinal Bacteria Reference Unit (GBRU) at Colindale, London who reported it as *Salmonella enterica* subspecies *enterica* serovar Newport on 6 December 2011. On 13 December 2011, the result was communicated through the Rapid Alert System for Food and Feed (RASFF) of the European Commission [1].

In late December 2011, Health Protection Scotland (HPS) reported four cases of S. Newport, all with the same pulsed-field gel electrophoresis (PFGE) profile which had not previously been seen. Concurrently in England, Wales and Northern Ireland, reporting of *S.* Newport infections exceeded expected levels. Molecular analysis of isolates from the human cases from all four countries indicated a PFGE profile indistinguishable from the sliced watermelon isolate.

On 13 January 2012, Germany reported through the Epidemic Intelligence Information System (EPIS) at the European Centre of Disease Prevention and Control (ECDC) fourteen *S.* Newport isolates that were indistinguishable from the PFGE profile of the sliced watermelon isolate. Four cases with this profile were also reported in Ireland in January 2012.

A multi-agency outbreak control team (OCT) was convened on 16 January 2012 comprising staff from PHE, Public Health Wales (PHW), HPS and the United Kingdom (UK) Food Standards Agency (FSA). There

were separate communications with the Robert Koch Institute (RKI) regarding the German cases and with the Health Protection Surveillance Centre (HPSC) and the National *Salmonella*, *Shigella* and *Listeria* Reference Laboratory (NSSLRL) regarding cases from Ireland. German and Irish public health and food safety authorities subsequently joined the OCT.

The aims of investigations were to gather and collate information on exposures, to identify the potential source(s), to institute immediate control measures and to determine if there were any lessons to be learnt regarding future prevention.

We describe an outbreak of *S.* Newport across six countries linked to the consumption of watermelon originating from Brazil.

Methods

Epidemiological investigations

Case ascertainment

Surveillance data from each of the six countries were reviewed for 2011. Exceedances in *S.* Newport cases were detected in December 2011. Therefore it was decided to perform pulsed-field gel electrophoresis (PFGE) analysis on all *S.* Newport cases identified from 31 October 2011 onwards to account for any outbreak cases that might have arisen in the weeks prior to the exceedance indicator being triggered.

Case definition

Outbreak cases were defined as persons with: (i) laboratory-confirmed infection with fully antimicrobial-sensitive *S.* Newport exhibiting the outbreak PFGE profile designated as SNEWXB.0110 (defined by the watermelon isolate); (ii) symptoms including diarrhoea or any two or more of: vomiting, fever or abdominal pain; (iii) onset of illness between 31 October 2011 and 31 January 2012; and (iv) who was reported in any of the six countries.

Food-borne illness questionnaire

In England, Wales and Northern Ireland, upon notification of *Salmonella* cases to PHE centre's (PHEC's), cases are routinely followed up by the PHEC and Environmental Health Officers (EHOs) in local authorities; Cases were contacted and asked to complete either the *Salmonella* or the generic food-borne illness questionnaire. While questionnaire formats varied between local authorities, all sought information on clinical, travel and food history in the seven days before illness. Specific questions around fruit consumption were not included. During the outbreak completed questionnaires were returned to epidemiologists in the Gastrointestinal, Emerging and Zoonotic Infections Department (GEZI), PHE, Colindale, where they were reviewed to elucidate commonality of exposures between cases.

Outbreak case exposure questionnaire

Following the first OCT meeting on 16 January 2012, a questionnaire was designed to collect information specifically on fruit consumption and was shared with each member country of the OCT. The questionnaire gathered detailed information on consumption of different types of fruit, including melon, and addressed whether products were pre-packaged, sliced, cubed, mixed with other fruit and where they were purchased and consumed. Cases in England, Wales, Northern Ireland and Ireland were interviewed by designated investigators using the bespoke questionnaire. Cases were not followed up if contact details were unobtainable. Attempted contact was made on a maximum of five occasions, at which point they were designated as being lost to follow-up.

The same questionnaire was used for cases in England, Wales, Northern Ireland and Ireland to interview cases. In Scotland, the questionnaire was completed retrospectively by investigators at HPS using information collected in interviews with EHOs. In Germany, cases were interviewed using a translation of the questionnaire supplied by the UK OCT with additional, more detailed questions on food history in the three days before disease onset.

Analytical comparison of watermelon consumption

To estimate the frequency of watermelon consumption in a group of persons in Germany unaffected by *S.* Newport infection, staff members included in the official mailing list of one of the RKI departments were invited via email to participate in an ad-hoc survey on 25 January 2012. The survey included the question whether any watermelon had been consumed in December 2011. The proportion of persons reporting watermelon consumption among interviewed outbreak cases (in the three days before symptom onset) and among RKI department staff members were compared. Odds ratios (OR) and corresponding 95% confidence intervals (CI) were calculated and statistical significance ($p<0.05$) was evaluated using Fisher's exact test. Data analysis was performed using Stata 12 (Stata Corporation, College Station, United States).

In the UK, the FSA extracted data on watermelon consumption from the UK National Diet and Nutrition Survey dataset for comparison with cases [2]. UK case consumption data were insufficient to support statistical analyses.

Microbiological investigations

Isolates from patients and food samples were characterised and compared. In England, Wales and Northern Ireland, all isolates were sent to GBRU; isolates in Scotland to the Scottish *Salmonella*, *Shigella*, and *Clostridium Difficile* Reference Laboratory; in Ireland to the NSSLRL and in Germany to the National Reference Centre for *Salmonella* and other Bacterial Enteric Pathogens at the RKI, Wernigerode.

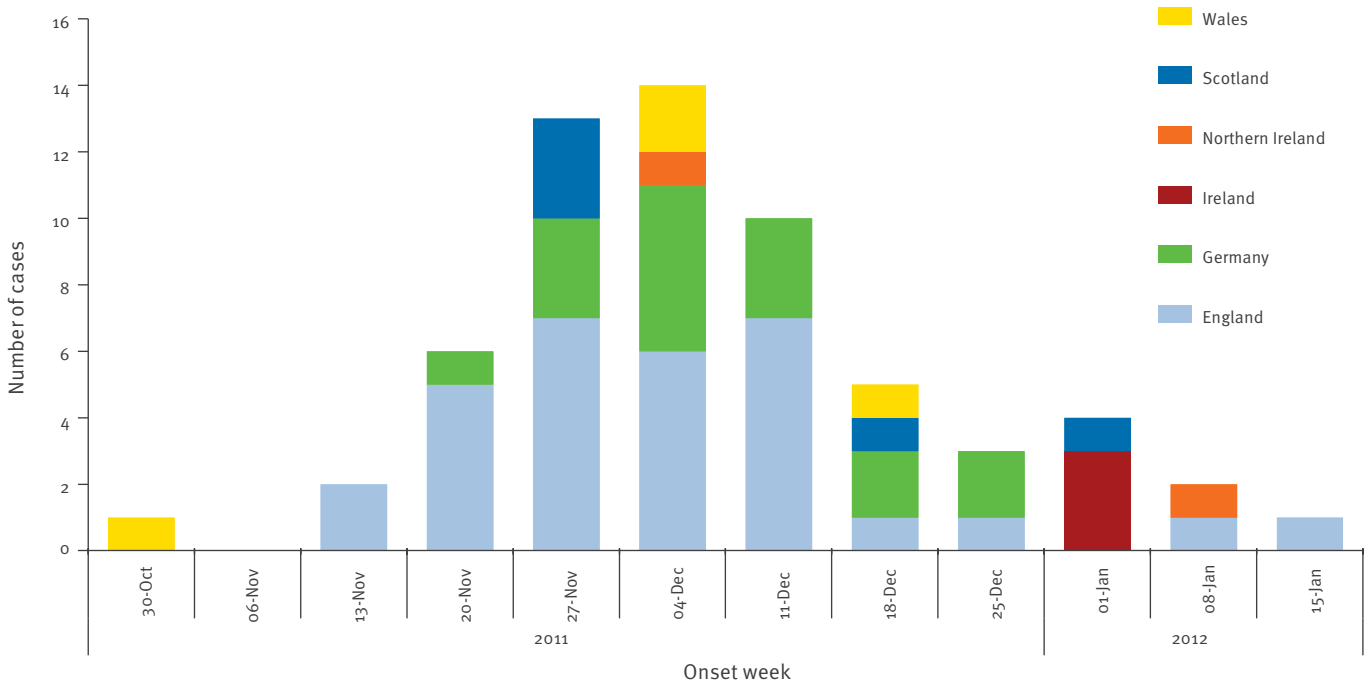
All isolates were serotyped according to the White-Kauffmann-LeMinor scheme [3]. Antimicrobial resistance profiles were determined by testing against a routine panel of antimicrobials in each Reference Laboratory. Each reference laboratory tested for ampicillin, cefotaxime, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, tetracyclines and trimethoprim/trimethoprim-sulfamethoxazole resistance as well as a range of additional antibiotics depending on the participating laboratory. Cut-off values according to the European Committee on Antimicrobial Susceptibility Testing (http://www.eucast.org) were used in Germany and Ireland. For isolates received by GBRU and in the Scottish reference laboratory, cut-off values were used as previously described on the basis of long-term studies [4-5].

PFGE using the PulseNet-Europe/Salm-gene protocol was performed on all *S.* Newport isolates from patients with an onset of illness date reported during the outbreak period (31 October 2011 to 31 January 2012) [6]. The PFGE profile from the watermelon isolate was designated the outbreak profile (SNWPXB.0110). Comparisons between profiles were analysed using algorithms within the BioNumerics software package (version 6.10; Applied Maths, Sint-Martens-Latem, Belgium). Dendrograms were constructed using the Dice similarity coefficient and the unweighted pair group method with arithmetic averages (UPGMA). The PFGE images of the outbreak strain were shared with PulseNet US and PulseNet Latin America. The results of PFGE analysis together with antimicrobial resistance profiles were used to inform case definitions.

A subset of isolates from UK cases were selected for whole genome sequencing (WGS). This included 24 of 37 outbreak isolates from UK cases and the isolate from the watermelon slice. An additional 11 non-outbreak isolates were selected for comparison. Genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega), and samples were sequenced using multiplex libraries on the HiSeq platform (Illumina) using 100 bp paired-end reads. The sequence data were aligned to the reference strain *S.* Newport SL254 (hereafter, SL254) along with its associated plasmids pSL254_3 and pSN254 (accession numbers CP001113, CP001112 and CP000604, respectively) using SMALT v0.6.4 [7]. Single nucleotide polymorphisms (SNPs) was compared to the reference strain and a maximum likelihood phylogeny of the isolates was constructed using RAxML [8-9]. A high divergence between the outbreak isolates and the reference SL254 was observed, with ca 50,600 SNPs separating the outbreak isolates from the reference SL254. To improve resolution, the outbreak isolates,

Figure 1

Epidemic curve based on onset week[a] of confirmed *Salmonella* Newport outbreak cases[b] in the United Kingdom, Ireland and Germany, 31 October 2011–22 January 2012 (n=61)



a For two cases in Wales and one in England, onset date was not known and specimen date was used.

b Excludes one asymptomatic case from Ireland and one German case where neither onset nor specimen date were reported. In total, 61 confirmed cases are included.

along with four non-outbreak isolates used as outliers, were re-mapped against the unordered assembly of the sliced watermelon isolate. De novo assembly of the watermelon isolate was undertaken using Velvet with a k-mer of 61, without scaffolding, giving an N50 of 301,843 and 192 nodes [10].

Traceback investigations

Following isolation of *S.* Newport from the watermelon slice, the FSA undertook product traceback. The Food Safety Authority of Ireland (FSAI) also performed traceback investigations into the source of watermelon consumed by a family cluster.

In Germany, traceback investigations were coordinated at the federal level by the Federal Institute for Risk Assessment (Bundesinstitut für Risikobewertung (BfR)). They started from five supermarket branches of one chain, where cases had purchased watermelons. Information on the immediate provider of watermelons was obtained through delivery documents provided by the stores to local veterinary offices. Watermelon deliveries were then further traced back stepwise by the federal states authorities and the BfR. The distribution chain was determined to originate from a single watermelon distributor in the Netherlands. The Federal Office of Consumer Protection and Food Safety (Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, (BVL)) in Germany then contacted food safety authorities in the Netherlands to obtain further information on the origin of the watermelons distributed by the Dutch company.

Results

Epidemiological investigations

Outbreak description

Between all six countries a combined total of 63 confirmed outbreak cases were reported with onset dates during the outbreak period (31 October 2011 to 31 January 2012): 31 in England, four in Wales, two in Northern Ireland, five in Scotland, four in Ireland, and 17 in Germany.

Onset dates ranged from 5 November 2011 to 19 January 2012, with almost half (29/61) of these between the 28 November and 11 December 2011 (Figure 1).

Cases were predominantly female (45/63) and ranged in age from six months to 95 years, with the greatest number (15/63) in children aged five years or less. In England, Wales and Northern Ireland, cases were distributed across all regions, with a predominance of cases in the east (7/37) and south-west (5/37) of England. In Germany, cases were reported from seven federal states across the country.

In total, 14 cases were hospitalised: three in England, two in Wales, one in Ireland and eight in Germany. There were three fatalities: one in England (aged 56 years) and two in Germany (aged 92 and 95 years); all three had underlying health conditions (cancer or cardiovascular disease).

Table

Reporting of watermelon consumption among confirmed cases of *Salmonella* Newport of the outbreak profile interviewed in the United Kingdom, Ireland and Germany, 31 October 2011–22 January 2012 (n=63)

Country	Number of outbreak cases	Number of cases interviewed	Reported watermelon consumption
England	31	19	13
Scotland	5	4	4
Wales	4	4	0
Northern Ireland	2	2	1
Ireland	4	4	3
Germany	17	13	6 [a]
All countries	**63**	**46**	**27**

[a] Two additional cases reported 'possible' consumption of watermelon.

Case exposures

In England, Wales and Northern Ireland, completed EHO questionnaires were received for 22 of 37 confirmed cases, including 11 who reported consumption of fruit and one who specified melon.

In total, 46 of 63 confirmed cases were interviewed using the outbreak case exposure questionnaire, including 19 cases from England, four from Wales, two from Northern Ireland four from Scotland, four from Ireland and 13 from Germany (Table). Of the remaining 17 cases not interviewed, three were deceased, five did not respond, contact details were unavailable for six, two refused consent to be interviewed and one had an onset date in early November and was not followed up due to the long time period which had elapsed.

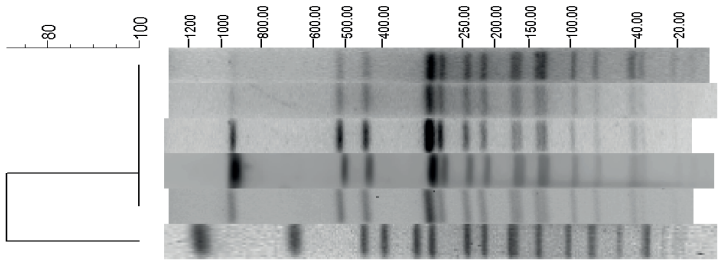
Consumption of pre-packed, pre-sliced or whole watermelon was reported by 59% of interviewed cases. In Ireland, three of the four cases were a family cluster who reported purchasing a whole melon which they sliced and consumed at home. The fourth case had no history of watermelon consumption.

An additional two cases in Germany reported possible consumption of watermelon. The remaining fifteen cases reported no consumption of watermelon, two of them reported consumption of other types of melon (Galia and Cantaloupe).

In follow-up interviews with cases from England and Northern Ireland, retailer information was reported for 21 confirmed cases. All four of the largest UK supermarket retailers were reported as well as independent green grocers, and their proportions were largely

Figure 2

Representative pulsed-field gel electrophoresis profile of *Salmonella* Newport isolates from confirmed outbreak cases (n=4) and the watermelon sample, United Kingdom, Ireland and Germany, 31 October 2011–22 January 2012



reflective of their market shares. In Scotland, one other retailer was cited by consumers, which was not reported by cases elsewhere in the UK. The German cases with confirmed watermelon consumption reported five different branches of one supermarket chain where they had purchased watermelon which was sliced in store.

Analytical comparison of watermelon consumption

Of 168 persons included in the RKI department staff emailing list, 91 (54%) replied. Of the persons who could remember whether they had consumed watermelon (n=87), seven (8%) affirmed consumption. This compares to 46% (6/13) of the German cases interviewed who remembered watermelon consumption (Table) and corresponds to an OR of 9.8 (95% CI: 2.6–37.3, two-sided p value ‹0.01).

Data on watermelon consumption extracted from the 2008–10 UK National Diet and Nutrition Survey dataset indicated that the level of reported watermelon consumption in the UK is normally 5–10% [9]. Consumption reported by cases (64%) was therefore considerably higher than would be expected in the general population.

Microbiological investigations

A single strain of *S.* Newport was found to be involved in this outbreak, with the novel PFGE profile SNWPXB.0110 (Figure 2). Retrospective analysis of *S.* Newport isolates detected in cases in England and Wales (n=91) before the outbreak period revealed no isolates with the same profile. Furthermore, no matches were found to this profile in PulseNet US or Latin America databases. All isolates were fully susceptible to the antimicrobials tested. WGS revealed identical sequences between the watermelon isolate and 19 of the 24 sequenced outbreak isolates from the UK. The remaining five sequenced isolates differed from the watermelon isolate by just one SNP. The non-outbreak strains differed by several thousand SNPs (Figure 3).

Traceback

In the UK, product follow-up of the contaminated watermelon slice indicated the product originated from a batch of watermelons imported from Brazil with a use-by date of 25 November 2011. The watermelons from which the slice was produced had been supplied whole to a major UK processor where they were sliced, packaged and distributed. *Salmonella* was not detected in subsequent raw material or packed product that was sampled at the processor's premises. As the use-by date of the affected batch had expired before the detection of *S.* Newport infections, no action could be taken to recall or withdraw the product. Further investigations were undertaken following human cases in Scotland in late December 2011. The confirmed cases in Scotland reported a different retailer from the one implicated in November, and traceback indicated a separate supply chain which did not involve the same UK processor. However, the melons were sourced from the same region in Brazil but from a different grower.

The FSAI performed a traceback on the watermelon consumed by the family cluster in Ireland. It had arrived in a shipment from Brazil and further work traced it back to the same source as the watermelon slice found positive for the outbreak strain in November 2011. The watermelons had been harvested on 25 October 2011, dispatched on 31 October 2011 and arrived in Dublin on 21 November 2011. The entire consignment was distributed between 1 December and 31 December 2011, consistent with the latest onset date in early January 2012. No action was necessary as the implicated product was no longer on sale in Ireland.

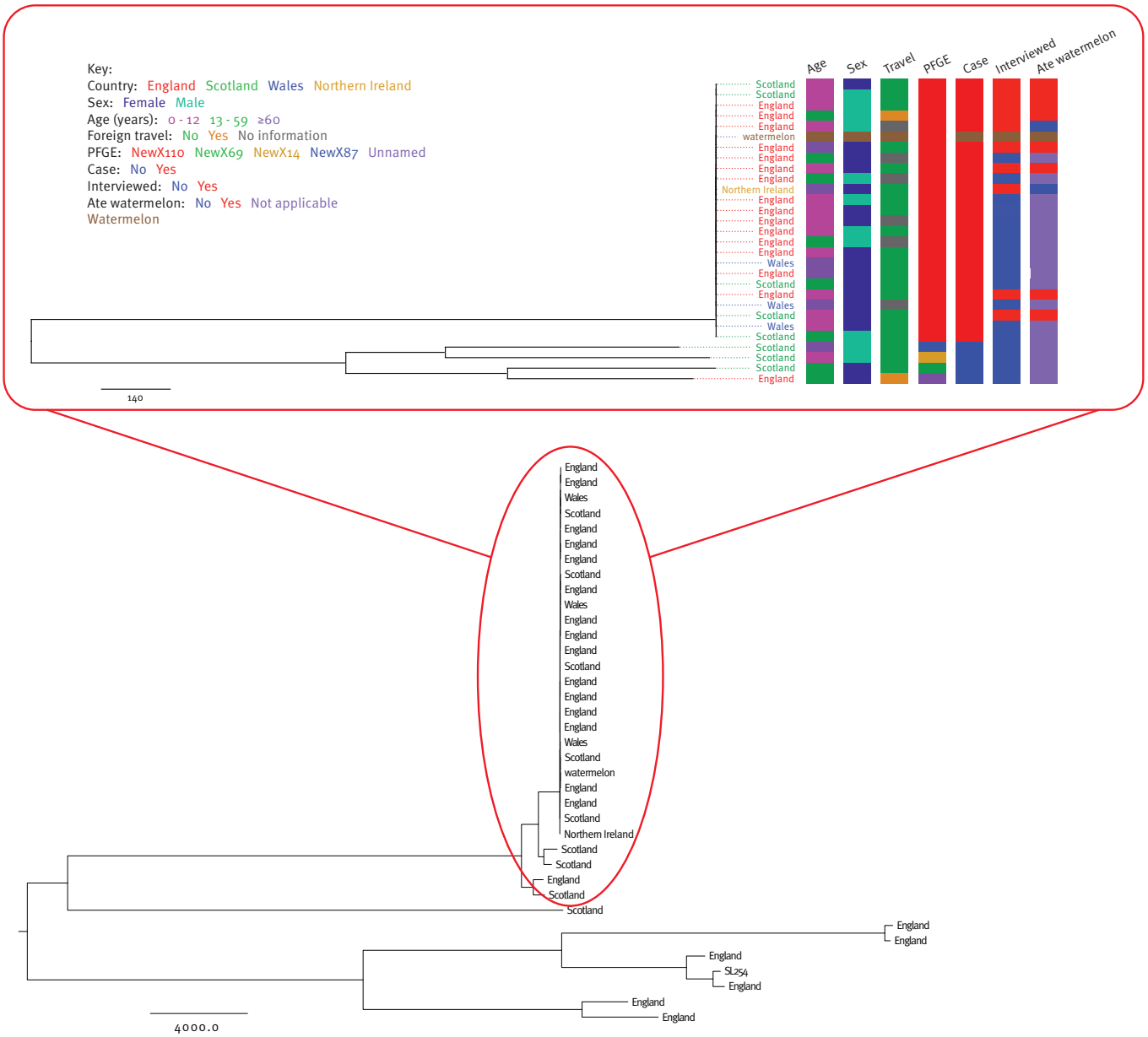
German authorities traced back watermelons from five supermarket branches of one chain where cases had reported purchasing watermelon, to a distributor in the Netherlands. From there, watermelons were traced back to the same grower in Brazil associated with the contaminated watermelon slice and the Irish cases and to an affiliated grower working together in a co-operative in the same geographical region in Brazil.

Discussion

Molecular, epidemiological, and traceback investigations identified a multi-country outbreak of *S.* Newport

Figure 3

Phylogeny of *Salmonella* Newport isolates from the sliced watermelon, confirmed outbreak cases (n=24) and non-outbreak cases (n=4), United Kingdom, Ireland and Germany, 31 October 2011–22 January 2012



S. Newport cases mapped to reference sequence SL254.

Inset: isolates mapped to the assembly of the sequence from the watermelon isolate. Scale bars represent divergence in the number of single nucleotide polymorphisms.

gastroenteritis linked to consumption of watermelon imported from Brazil.

Molecular analysis determined the contaminated watermelon strain and human isolates from confirmed cases in the UK, Ireland and Germany indistinguishable from one another by PFGE and, for a subset, by WGS. In this outbreak investigation, epidemiological studies were successfully informed by analyses of PFGE data. The source of infection was confirmed through standard food traceback investigations. We used this opportunity to examine and evaluate the use of WGS for the purposes of food-borne outbreak investigation. We found that using WGS data, we were able to describe with greater clarity the relatedness of strains isolated from cases and watermelons and also demonstrate the scale of difference between these organisms and other *S*. Newport strains concurrently circulating in Europe. While it could be deduced from the traceback investigations that the outbreak strain was not European in origin, the WGS data provided objective evidence to confirm the scale of genetic difference between

imported and indigenous strains. Understanding these effects should help inform future food-borne outbreak investigations where WGS data analyses could be used to provide important clues as to the geographic origins of implicated food vehicles, which in turn could feed back into hypothesis generation.

As costs decline, and the speed and fidelity of sequencing improve, WGS is becoming an increasingly accessible element of public health microbiology. It has been postulated that WGS may soon replace conventional typing in the investigation and management of outbreaks [11]. WGS can provide a level of evidence of linkage between specific sources of infection and human cases that is close to irrefutable. In view of the sensitivity of reputation for food business operators and food-producing countries, this level of evidence is important in gaining support for intervention measures to protect public health. Furthermore, as genome databases of major food-borne pathogens expand it is reasonable to expect detection of previously unrecognised links between cases of infection and complex food chains. The challenge for public health microbiology laboratories is to achieve the capacity for rapid sequence determination, data storage and analysis that will allow full exploitation of this powerful new approach [12].

Traceback investigations identified Brazilian watermelons as a common factor in cases associated with the outbreak. Consumption of watermelon was reported by a high proportion of cases across all six countries as compared to the general population [2]. The German convenience sample also indicated a higher level of consumption of watermelon in outbreak cases than their controls. However, recall and selection bias may have led to some underestimation of consumption in the study population.

Cases occurred over a number of weeks and, as identified by traceback, storage and distribution of watermelon spans several weeks. Cases declined in February, coinciding with the end of the Brazilian watermelon importation season. The UK Department for Environment, Food and Rural Affairs Statistics team and the Rural Payment Agency's Horticultural Marketing Inspectorate indicated that the season for imported Brazilian watermelons runs from August to February in the UK.

The same melon grower was associated with the contaminated watermelon slice and human cases in Ireland and Germany. The melons associated with the Scottish cases were traced back to a different grower in the same region. However, the isolates from Scottish cases shared a common sequence with the other cases, therefore it is likely that Brazilian watermelons from a particular area were the vehicle, but that these were not confined to one specific grower.

Cases reported consumption of watermelons whole, pre-packaged and sliced, alone, or as part of fruit salads or melon mixtures, indicating the melons themselves were contaminated and contamination did not occur during processing. Furthermore, different retailers, with different importers, processors and distributors were reported, also precluding these as the source of contamination. It is not possible to confirm at what point contamination occurred but there is likely to have been a common source of *S.* Newport which contaminated the produce at an unidentified point between growing and distribution.

In the United States (US) and Canada, melons have consistently been linked to outbreaks of *Salmonella* [13-17]. Three multistate outbreaks of *S.* Poona infection associated with eating cantaloupe melons imported from Mexico occurred in three consecutive years during the spring of 2000, 2001 and 2002 [18]. In 2011, in the US, a multistate outbreak of *S.* Panama linked to cantaloupe melons harvested in Guatemala occurred between February and April [19]. Outbreaks of salmonellosis linked to melons have also occurred in Australia in 2006 and New Zealand in 2009 [20-21].

This is the first outbreak of salmonellosis in Europe associated with melons from South America. Given that this is a ready-to-eat product, and that *salmonella*e have the ability to attach to or enter into vegetables and fruits, it is likely that these produce items are potential sources for future outbreaks of salmonellosis [15]. The watermelon market has been developed by Brazil in recent years and Brazilian exports to the UK have been around 6,000 tonnes per annum in recent years. Brazil also exports similar quantities to Germany and the Netherlands [data not shown].

Recurrent outbreaks such as the one described here highlight the importance of good agricultural practices and hygiene manufacturing controls from the grower to the consumer. As a consequence of repeated RASFF notifications dealing with contaminated watermelons from Brazil, 10% of shipments of watermelons imported from Brazil into the European Union have been tested for *Salmonella* since January 2013 [22].

The use of standardised case exposure questionnaires and the use of PFGE and WGS to recognise outbreaks more easily, would improve both their timely detection and control. Our use of genome sequencing information and PFGE demonstrated the ability of these techniques to unequivocally link cases which would have been impossible to connect using limited available traceback information. Informal (PulseNet International, EPIS) and formal (European Early Warning and Response System and RASFF) communication platforms enabled the full geographical extent of the outbreak to be rapidly recognised and exemplify their use as valuable media for informing public health experts. International trade in food will always provide the opportunity for infections to appear far from the source of contamination. We have the tools to identify and respond to such outbreaks and their use should be maximised.

Acknowledgements

In addition to all members of the outbreak control team, the OCT in Germany would like to thank local and state health and food safety authorities for their support. We would like to thank microbiologists and staff from the HPA Gastrointestinal Bacteria Reference Unit and the Scottish *Salmonella*, *Shigella* and *Clostridium difficile* Reference Laboratory and also the patients for their collaboration in providing data.

The sequencing work in this paper was supported by Wellcome Trust grant WT076964 through funding from The Wellcome Trust Sanger Institute

Conflict of interest

None declared.

Authors' contributions

LB: managed data collection and collation for cases in England, Wales and Northern Ireland and wrote the draft manuscript. IF: coordinated the local public health response, contributed to data collation, read and revised the draft manuscript and approved the final version. TP: was responsible for molecular analysis and interpretation of laboratory results for isolates from England, Wales and Northern Ireland, reviewed the manuscript and approved the final version. AM and NT: Designed and performed phylogenetic analysis and provided results, contributed to drafting the manuscript, made revisions, and approved the final version. HB and BR: were the principle investigators in Germany and made substantial contributions to study conception and design, data collection, analysis and interpretation. Both critically revised the manuscript and approved the final version. PM: collected data in Ireland and coordinated the Irish public health response. MC performed laboratory testing in Ireland, read and revised the manuscript. JC: collected and co-ordinated data from Scotland and contributed to the public health response. VA assisted in study design and undertook data collection. CL contributed to study design, coordinated the initial UK public health response, and critically reviewed the manuscript.

International Outbreak Control Team

Joanne Aish, Goutam Adak, Victor Aiyedun, Derek Brown, Lynda Browning, Paul Cook, Cecilia Ellis, Andrew Fox, Elizabeth de Pinna, Patricia Garvey, Kathie Grant, Dorothy Guina-Dornan, Petra Hiller, Colin Houston, Naomi Launders, Burkhard Malorny, Brendan O'Brien, Beatrice Pfefferkorn, Rita Prager, Wolfgang Rabsch, Roland Salmon, Robert Smith, Brian Smyth, Kirsten Stone and Heidi Wichmann-Schauer.

References

1. Rapid Alert System for Food and Feed (RASFF). Brussels: European Commission. [Accessed: 1 Aug 2014]. Available from: http://ec.europa.eu/food/safety/rasff/portal/index_en.htm
2. Whitton C, Nicholson SK, Roberts C, Prynne CJ, Pot GK, Olson A, et al. National Diet and Nutrition Survey: UK food consumption and nutrient intakes from the first year of the rolling programme and comparisons with previous surveys. Br J Nutr. 2011:106(12):1899-914. http://dx.doi.org/10.1017/S0007114511002340
3. Grimont PAD, Weill FX. Antigenic formulas of the *Salmonella* serovars. 9th ed. Paris: World Health Organization Collaborating Centre for Reference and Research on *Salmonella*, Institut Pasteur; 2007. Available from: http://www.pasteur.fr/ip/portal/action/WebdriveActionEvent/oid/01s-000036-089
4. Frost JA. Testing for resistance to antimicrobial drugs. In: Chart H, editor. Methods in practical laboratory bacteriology. New York: CRC Press; 1994. pp. 73-82.
5. Browning LM, Brown DJ, Coia JE, Cowden JM, Mather H. Antimicrobial resistance of *Salmonella* in Scotland, 2005 (excluding S. Typhi and S. Paratyphi). HPS Weekly Report. 2007;21 (41):172. Accessed 1 Aug 2014. Available from: http://www.documents.hps.scot.nhs.uk/ewr/pdf2007/0721.pdf
6. Peters TM, Berghold C, Brown D, Coia J, Dionisi AM, Echeita A, et al. Relationship of pulsed-field profiles with key phage types of *Salmonella enterica* serotype Enteritidis in Europe: results of an international multi-centre study. Epidemiol Infect. 2007;135(8):1274-81. http://dx.doi.org/10.1017/S0950268807008102
7. Wellcome Trust Sanger Institute (WTSI). SMALT. Hinxton: WTSI. [Accessed: 1 Aug 2014]. Available from: http://www.sanger.ac.uk/resources/software/smalt/
8. Harris SR, Feil EJ, Holden MT, Quail MA, Nickerson EK, Chantratita N, et al. Evolution of MRSA during hospital transmission and intercontinental spread. Science. 2010;327(5964):469-74. http://dx.doi.org/10.1126/science.1182395
9. Stamatakis A. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics. 2006;22(21):2688-90. http://dx.doi.org/10.1093/bioinformatics/btl446
10. Zerbino DR, Birney E. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res. 2008;18(5):821-9. http://dx.doi.org/10.1101/gr.074492.107
11. Sabat AJ, Budimir A, Nashev D, Sá-Leão R, van Dijl JM, Laurent F, et al. Overview of molecular typing methods for outbreak detection and epidemiological surveillance. Euro Surveill. 2013;18(4):pii=20380
12. Achtman M, Wain J, Weill F-X, Nair S, Zhou Z, Sangal V, et al. Multilocus Sequence Typing as a Replacement for Serotyping in *Salmonella* enterica. PLoS Pathog. 2012;8(6):e1002776. http://dx.doi.org/10.1371/journal.ppat.1002776
13. Bowen A, Fry A, Richards G, Beuchat L. Infections associated with cantaloupe consumption: a public health concern. Epidemiol Infect. 2006;134(4):675-85. http://dx.doi.org/10.1017/S0950268805005480
14. Gayler GE, MacCready RA, Reardon JP and McKernan BF. An outbreak of salmonellosis traced to watermelon. Public Health Rep. 1955:70(3):311-3. http://dx.doi.org/10.2307/4589055
15. Hanning IB, Nutt JD, Ricke SC. Salmonellosis outbreaks in the United States due to fresh produce: sources and potential intervention measures. Foodborne Pathog Dis. 2009;6(6):635-48. http://dx.doi.org/10.1089/fpd.2008.0232
16. Blostein J. An outbreak of *Salmonella* Javiana associated with consumption of watermelon. J Environ Health. 1993;56(1):29-31.
17. Centers for Disease Control and Prevention (CDC). Foodborne outbreak online database (FOOD). Atlanta: CDC. [Accessed: 1 Aug 2014]. Available from: http://wwwn.cdc.gov/foodborneoutbreaks/Default.aspx
18. Centers for Disease Control and Prevention (CDC). Multistate outbreaks of *Salmonella* serotype Poona infections associated with eating cantaloupe from Mexico--United States and Canada, 2000-2002. MMWR Morb Mortal Wkly Rep. 2002;51(46):1044-7.
19. Centers for Disease Control and Prevention (CDC).Investigation update: Multistate outbreak of *Salmonella* Panama infections linked to cantaloupe. Atlanta: CDC; 2011. Available from: http://www.cdc.gov/*salmonella*/panama0311/062311/index.html
20. Munnoch SA, Ward K, Sheridan S, Fitzsimmons GJ, Shadbolt CT, Piispanen JP, et al. A multi-state outbreak of *Salmonella* Saintpaul in Australia associated with cantaloupe consumption. Epidemiol Infect. 2009;137(3):367-74. http://dx.doi.org/10.1017/S0950268808000861
21. McCallum L, Torok M, Dufour MT, Hall A, Cramp G. An outbreak of *Salmonella* Typhimurium phage type 1 associated with watermelon in Gisborne, January 2009. N Z Med J. 2010;123(1322):39-45.
22. European Commission. Commission Implementing Regulation (EU) No 1235/2012 of 19 December 2012 amending Annex I to Regulation (EC) No 669/2009 implementing Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards the increased level of official controls on imports of certain feed and food of non-animal origin. Brussels: European Commission; 2012. L 350/44-50. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2012:350:0044:0050:EN:PDF

Epidemiology and outcome of invasive pneumococcal disease among adults in Belgium, 2009–2011

J Verhaegen (jan.verhaegen@uzleuven.be)[1], J Flamaing[2], W De Backer[3], B Delaere[4], K Van Herck[5,6], F Surmont[7], Y Van Laethem[8], P Van Damme[5], W Peetermans[9]

1. Department of Microbiology, University Hospitals Leuven, KU Leuven, Belgium
2. Department of Geriatric Medicine, University Hospitals Leuven, KU Leuven, Belgium
3. Department of Respiratory Medicine, University Hospital Antwerp, Antwerp, Belgium
4. Infectious Diseases Department, Cliniques Universitaires UCL de Mont- Godinne, Yvoir, Belgium
5. Vaccine & Infectious Disease Institute, University of Antwerp, Antwerp, Belgium
6. Department of Public Health, Ghent University, Ghent, Belgium
7. Medical Department, Specialty Care Business Unit Pfizer, Brussels, Belgium
8. Department of Infectious Diseases, St Pierre Hospital, Brussels, Belgium
9. Department of Internal Medicine and Infectious Diseases, University Hospitals Leuven, KU Leuven, Belgium

Citation style for this article:
Verhaegen J, Flamaing J, De Backer W, Delaere B, Van Herck K, Surmont F, Van Laethem Y, Van Damme P, Peetermans W. Epidemiology and outcome of invasive pneumococcal disease among adults in Belgium, 2009–2011. Euro Surveill. 2014;19(31):pii=20869. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20869

Article submitted on 19 August 2013 / published on 07 August 2014

This epidemiological study examined morbidity and case fatality of invasive pneumococcal disease (IPD) in adults in Belgium as well as distribution and antibiotic susceptibility of *Streptococcus pneumoniae* serotypes. Adults hospitalised with microbiologically proven IPD were prospectively enrolled. The study started in 2009 with patients aged ≥50 years, whereas in 2010 and 2011, patients aged ≥18 years were included. The clinical presentation, patient profile, treatment, outcome, and mortality were recorded during hospitalisation. Outcome was also assessed one month after discharge. Of the 1,875 patients with IPD identified, 1,332 were included in the analysis. Bacteraemic pneumonia, affecting 1,049 of the patients, was the most frequent IPD type (79%), and chronic obstructive pulmonary disease and cancer were the main comorbidities. One-third of patients required admission to intensive care unit. A total of 208 (16%) patients died during hospitalisation and an additional 21 (2%) within one month after discharge. Case fatality rates of ≥20% were observed in patients with chronic heart failure, hepatic disease, and renal insufficiency. Serotypes 7F, 1, 19A, and 3 were the most prevalent and together accounted for 47% (569/1,214) of all IPD cases and 42% (80/189) of mortality. Of the patient isolates, 21% (255/1,204) were resistant to erythromycin and 22% (264/1,204) to tetracycline. Penicillin non-susceptibility was mostly found in serotype 19A isolates. These baseline data are essential when assessing the impact of pneumococcal conjugate vaccination in adults in the future.

Introduction

In industrialised countries, the risk of invasive pneumococcal disease (IPD) remains high among older adults despite the availability of the 23-valent pneumococcal polysaccharide vaccine (PPV23) since 1983 [1]. In a large number of these countries, including Belgium, PPV23 is recommended since 1985 for all adults ≥65 years of age and for persons between two and 64 years-old at high risk for pneumococcal infections due to living conditions or underlying medical conditions including asplenia, human immunodeficiency virus (HIV) infection, immunodeficiency or chronic cardiac, pulmonary, renal or hepatic diseases as from 50 years of age [2,3]. In 2004, the 7-valent pneumococcal conjugate vaccine (PCV7) for infant vaccination became available in Belgium in a 3+1 schedule at full charge of the parents and, in 2007, PCV7 was added free of charge to the universal infant vaccination programme in a 2+1 schedule at two, four and 12 months of age. Since September 2011, PCV7 has been replaced by the 13-valent pneumococcal conjugate vaccine (PCV13) in the Belgian childhood vaccination schedule. In 2011, PCV13 was also approved by the European Medicines Agency for the prevention of IPD in adults ≥50 years of age [4]. The Belgian recommendations regarding pneumococcal vaccination in adults were updated in July 2013 to also include PCV13 [2], however, there is no publicly funded pneumococcal vaccination programme for adults in Belgium.

A national IPD surveillance programme has existed in Belgium since 1986. It monitors the number of cases for all ages, type of IPD, serotypes or serogroups, and antibiotic susceptibility, but only few clinical data [5]. An active IPD surveillance network for young children started in Belgium in 2002 [6,7]. It showed that, two years after implementation of PCV7 in children <2 years of age, the incidence of vaccine-serotype IPD declined by 96% in this population but that the incidence of non-vaccine-serotype IPD increased two to three-fold [6]. Because the clinical data to assess the burden of disease in adults were lacking, a prospective, active, hospital-based study was started in 2009 to analyse the morbidity and case fatality rate of IPD in adults

aged ≥18 and ≥50 years, the distribution of pneumococcal serotypes and their antibiotic susceptibility, and the factors affecting disease outcome. We report the results for three years (2009–2011) of this study to document the epidemiology and the burden of IPD before the introduction of PCV13.

Methods

Study design

This is a prospective, active, hospital-based epidemiological study of IPD in adults in Belgium. Fifty hospitals participated, corresponding to 44% of the acute care hospitals in Belgium. Three of these hospitals provided data for only the first year of the study, five during two years, and 42 during the three-year study period.

Adults hospitalised with microbiologically confirmed IPD (defined as isolation of *Streptococcus pneumoniae* from a normally sterile body site such as blood or cerebrospinal fluid) were eligible for inclusion. During the first year of study (2009), only adults aged ≥50 years were included, but as of 2010, the study was extended to all adults aged ≥18 years. The patient or a legal representative gave an informed consent for inclusion. If no informed consent was obtained, the patient was considered as a screen failure and clinical data were excluded from the analysis. The study was approved by the institutional review boards and local ethics committees of the participating hospitals.

General baseline information was collected at inclusion, including detailed demographics, type of IPD, laboratory data, relevant medical history, and previous vaccination against *S. pneumoniae* and seasonal influenza. The clinical presentation, complications, diagnostic procedures, and treatment were documented by the treating physician during hospital stay. The disease outcome and persisting symptoms and signs were documented at discharge and one month after discharge. All patients were managed according to the hospital's standard protocol for IPD.

Microbiology

Pneumococcal culture was carried out by the clinical microbiology laboratory of each hospital using routine techniques. Pneumococcal isolates inoculated on blood agar plates or tubes were sent to the Belgian National Reference Laboratory for Pneumococci (University of Leuven, Belgium) for capsular typing and antibiotic susceptibility testing.

Serotyping of pneumococcal isolates was done by phase-contrast microscopy using the Quellung reaction with serotype/serogroup-specific sera obtained from the Statens Serum Institute (Copenhagen, Denmark). Antibiotic susceptibility was assessed using the disk diffusion method on Mueller Hinton blood agar plates: penicillin (oxacillin, 1 µg), erythromycin (15 µg), tetracycline (30 µg), and ofloxacin (5 µg). Isolates were categorised as fully susceptible, intermediately resistant, or resistant according to the interpretive standards (document M100-S22) of the Clinical and Laboratory Standards Institute (Wayne, PA) [8].

For isolates with oxacillin zone diameters ≤19 mm, penicillin minimum inhibitory concentrations (MICs) were determined with Epsilometer (E)-test on Mueller Hinton blood agar plates. For the interpretation of penicillin MIC results, criteria for parenteral penicillin (non-meningitis) were used.

Statistical analysis

Calculations and statistical analyses were performed with SAS (version 9.3 for Windows) statistical package (SAS Institute, Cary, NC). Quantitative variables were expressed as means and standard deviations (SD) or as medians and interquartile ranges (IQR). Categorical findings were summarised in frequency tables. Mean values were compared by one way analysis of variance, whereas proportions were compared using the chi-squared or Fisher's exact test. The association between outcome at discharge and covariates (age, IPD type, comorbidities, and vaccination status) was assessed by univariate and multivariate ordinal logistic regression and was expressed as an odds ratio with a 95% confidence interval (95% CI). In general, 'age' was preferred to age categories. A two-tailed p-value <0.05 was considered statistically significant.

Results

Participants

A total of 1,875 patients hospitalised with IPD were eligible. Informed consent was not obtained for 467 patients and, therefore, clinical data from these patients were excluded from analysis. In most cases, the informed consent was not obtained because of the patient's poor physical condition or because the patient was discharged before microbiological confirmation of IPD. Average age and sex ratio of these patients were similar to the analysed population (data not shown).

To avoid inclusion of nosocomial IPD cases, 76 IPD cases were also excluded because the interval between hospitalisation and blood draw was ≥5 days. Thus, 1,332 patients were included in the analysis, with only 220 of them being between 18 and 49 years of age partly because patients in this age group were only included during the last two years of the study. A total of 208 patients died during hospitalisation. Of the 1,124 patients who were discharged from hospital, 141 (13%) were lost to follow-up so that one-month follow-up results were analysed in 983 patients.

Of the total 1,332 patients included in the analysis 52% were male and the mean age was 66 years (range: 18–98). Three age groups comprising 18 to 49 year-olds, 50 to 64 year-olds and those aged ≥65 years were considered for the study, however in some analyses patients aged ≥50 years were compared to those aged 18 to 49 years. Baseline characteristics for the three age

Table 1

Baseline characteristics of patients with invasive pneumococcal disease by age group, Belgium, 2009–2011 (n=1,332)

Characteristics	Age group, n (%)			P value
	18–49 years (n=220)	50–64 years (n=370)	≥65 years (n=742)	
Sex				
Female	110 (50)	176 (48)	348 (47)	0.72
Male	110 (50)	194 (52)	394 (53)	
Living condition				
At home	218 (99)	361 (98)	621 (84)	<0.0001
In nursing home or other care centre	1 (<1)	7 (2)	116 (16)	
Unknown	1 (<1)	2 (<1)	5 (<1)	
Comorbidities[a]				
Any	118 (54)	274 (74)	627 (85)	<0.0001
Chronic obstructive pulmonary disease	15 (7)	89 (24)	231 (31)	<0.0001
Cancer	13 (6)	81 (22)	203 (27)	<0.0001
Heart failure	3 (1)	40 (11)	206 (28)	<0.0001
Diabetes	11 (5)	47 (13)	146 (20)	<0.0001
Renal insufficiency	3 (1)	32 (9)	144 (19)	<0.0001
Immunosuppression	21 (10)	51 (14)	100 (14)	0.32
≥ 2 comorbidities per patient	50 (23)	154 (42)	399 (54)	<0.0001
Previous vaccination against *Streptococcus pneumoniae*				
Yes	10 (5)	20 (5)	62 (8)	0.0023
No	179 (81)	242 (65)	401 (54)	
Unknown	31 (14)	108 (29)	279 (38)	
Previous vaccination against influenza				
Yes	18 (8)	79 (21)	326 (44)	<0.0001
No	181 (82)	230 (62)	278 (38)	
Unknown	21 (10)	61 (17)	138 (19)	
Oral antibiotics within 24 hours before admission				
Yes	14 (6)	15 (4)	37 (5)	0.46
No	206 (94)	354 (96)	705 (95)	
Unknown	0 (0)	1 (<1)	0 (0)	

[a] Only comorbidities found in more than 10% of the patients are shown.

groups are described in Table 1. The number of cases increased with age and the majority were ≥65 years-old. Comparing the number of cases per year per age group (110 in the 18–49 years, 121 in the 50–64 years and 237 in the ≥65 years) with the size of the population in Belgium per age group at the time of the study (4.6 million 18–49 year-olds, 2 million 50–64 year-olds and 1.8 million ≥65 year-olds), there appeared to be almost three times more cases in the 50 to 64 years age group compared to the 18 to 49 years, and almost six times more cases in the ≥65 year-olds.

Most patients had at least one chronic comorbidity, and the proportion increased from 54% (118/220) in patients aged between 18 and 49 years to 85% (627/742) in patients aged ≥65 years. Furthermore, 45% (603/1,326) of patients had ≥2 predisposing comorbidities. Chronic obstructive pulmonary disease and cancer were the most frequent comorbidities in the two older age groups. Even though the vast majority of patients had a comorbidity or were at an age where pneumococcal vaccination is recommended, less than 10% (92/1,332) were vaccinated with PPV23. Vaccination against seasonal influenza increased with increasing age, from 8% (18/220) in patients aged between 18 and 49 years to 44% (326/742) in patients aged ≥65 years. Nearly 5% (66/1,332) of patients took oral antibiotics within 24 hours before hospitalisation.

Type and outcome of invasive pneumococcal disease

Of the 1,332 patients, 1,049 (79%) had bacteraemic pneumonia (Table 2). Patients aged between 18 and 49 years were hospitalised for a median duration of 7.5 days (IQR: 5–13) compared to 12 days (IQR: 7–22) for patients aged ≥50 years. Admission to an intensive care unit (ICU) was more frequent in older patients (42% (154/370) in 50–64 year-olds vs. 25% (54/219) in 18–49

Table 2

Distribution of invasive pneumococcal disease types by patient age, Belgium, 2009–2011 (n=1,332)

Type of IPD	Total (n=1,332)	Age group, n (%)		
		18–49 years (n=220)	50–64 years (n=370)	≥65 years (n=742)
Bacteraemic pneumonia	1,049 (79)	170 (77)	276 (75)	603 (81)
Empyema	94 (7)	21 (10)	32 (9)	41 (6)
Meningitis	73 (6)	8 (4)	32 (9)	33 (4)
Bacteraemia without focus[a]	73 (6)	8 (4)	17 (5)	48 (7)
Other (e.g. septic arthritis, endocarditis, or peritonitis)	43 (3)	13 (6)	13 (4)	17 (2)

IPD: invasive pneumococcal disease.

[a] *Streptococcus pneumoniae* isolated from blood culture without localised infection identified.

Table 3

Admission to intensive care unit and disease outcome at discharge by age and type of invasive pneumococcal disease, Belgium, 2009–2011 (n=1,329)[a]

Category	n	Admission to ICU n (%)	Outcome at discharge			Univariate OLR	
			Cured n (%)	Discharged with persisting symptoms n (%)	Death n (%)	Odds ratio (95% CI)	Overall p value
Total[a]	1,329	434 (33)	884 (67)	237 (18)	208 (16)	–	–
Age in years							
18–49	219	54 (25)	157 (72)	49 (22)	13 (6)	1	0.044
50–64	370	154 (42)	240 (65)	83 (22)	47 (13)	1.42 (0.98–2.04)	
≥65	740	226 (31)	487 (66)	105 (14)	148 (20)	1.52 (1.10–2.12)	
Type of invasive pneumococcal disease							
Bacteraemic pneumonia	1,049	303 (29)	722 (69)	169 (16)	155 (15)	1	0.0049
Empyema	94	49 (52)	49 (52)	35 (37)	10 (11)	1.61 (1.07–2.45)	
Meningitis	73	59 (81)	38 (52)	16 (22)	19 (26)	2.06 (1.31–3.25)	
Bacteraemia without focus	73	12 (16)	51 (70)	4 (6)	18 (25)	1.12 (0.69–1.84)	
Other (e.g. septic arthritis, endocarditis, or peritonitis)	43	11 (26)	24 (56)	13 (30)	6 (14)	1.53 (0.84–2.79)	

CI: confidence interval; ICU: intensive care unit; OLR: ordinal logistic regression; SD: standard deviation.

[a] Data missing for three patients.

year-olds; p=0.001) and for patients with meningitis (81% (59/73); p=0.0001) (Table 3). Only 16% (12/73) of patients with bacteraemia without focus were admitted to ICU. Depending on the age group, the median durations of stay in an ICU varied from four to six days (IQR: 2–15), with four days for the 18 to 49 year-olds, six days for the 50 to 64 year-olds and five days for those aged ≥65 years, The median durations of hospitalisation after discharge from ICU were seven to 10.5 days (IQR: 0–21), with seven days for the 18 to 49 year-olds, nine days for the 50 to 64 year-olds and 10.5 for those 65 years-old and over. The median duration of ICU stay was significantly higher for deceased patients (9 vs. 5 days, p=0.0009).

For empirical treatment before the microbiological results became available, the three most used antibiotics on patients with available data (n=1,327) were amoxicillin-clavulanic acid intravenous (in 615 (46%) patients), third-generation cephalosporin (in 116 (9%) patients), and fluoroquinolones (in 71 (5%) patients). Also, 65 (5%) patients received a combination of amoxicillin-clavulanic acid and macrolides. After microbiological diagnosis, antibiotic treatment was changed to amoxicillin in 142 patients and penicillin in 151 patients. Overall, the antibiotic treatment was adapted in 55% (723/1,327) of patients after the microbiological results became available. The majority of the 73 patients with meningitis were treated with third-generation cephalosporin (51 patients) or penicillin (16 patients).

During hospitalisation 16% (208/1,329) of patients died. Duration of hospitalisation was significantly lower in deceased patients (median, 9 vs. 11 days; p=0.0013).

Of the 1,329 patients with outcome data at discharge, 884 (67%) were cured and 237 (18%) had persisting

symptoms and signs, including 18 with persistent fatigue (8%), 16 with pleural pain (7%), 49 with dyspnoea (21%), and 86 with pleural infiltrate (36%). Persisting symptoms and signs were less common in patients with bacteraemia without focus than in patients with other types of IPD, and patients with empyema had the highest rate of persisting symptoms and signs. Of the 983 patients with available one-month follow-up data, an additional 21 (2%) patients died within one month of discharge, 749 (76%) patients were cured, and 213 (22%) still had persisting symptoms and signs. The case fatality rate at discharge was highest for meningitis, lowest for empyema, and increased with increasing age (from 6% (13/219) in patients aged 18–49 years to 20% (148/740) in those aged ≥65 years; p<0.0001). The case fatality rate for meningitis decreased with increasing age (from 37% (3/8) in patients aged 18–49 years to 18% (6/33) in patients aged ≥65 years), whereas the case fatality rate of bacteraemia without focus increased (from 0% (0/8) in patients aged 18–49 years to 31% (15/48) in patients aged ≥65 years). A case fatality rate higher than 20% was seen for patients with comorbidities such as asplenia, alcoholism, renal insufficiency, hepatic disease, or heart failure (Table 4). Multivariate logistic regression showed that the risk of death was higher for patients with heart failure, renal insufficiency, and alcoholism but not for patients with chronic obstructive pulmonary disease, immunosuppression, or cancer. For patients admitted to ICU, case fatality rate was 22% (12/54) in patients aged <50 years and 32% (122/380) in patients aged ≥50 years.

Pneumococcal serotype distribution and antibiotic susceptibility

Of the 1,214 serotypes identified in isolates, serotypes 7F (159 isolates (13%)) serotypes 1 (154 isolates (13%)), serotypes 19A (145 isolates (12%)), and serotypes 3 (111 isolates (9%)) accounted for nearly half of IPD cases in adults (Figure 1). Serotype 1 was more prevalent in patients aged between 18 and 49 years (27% (54/200), p<0.0001), whereas serotype 3 was more prevalent in those aged ≥65 years (13% (84/669), p<0.0001). Serotype 7F was the most prevalent in patients with

Table 4

Disease outcome at discharge for patients with invasive pneumococcal disease, by comorbidity, Belgium, 2009–2011 (n=1,329)[a]

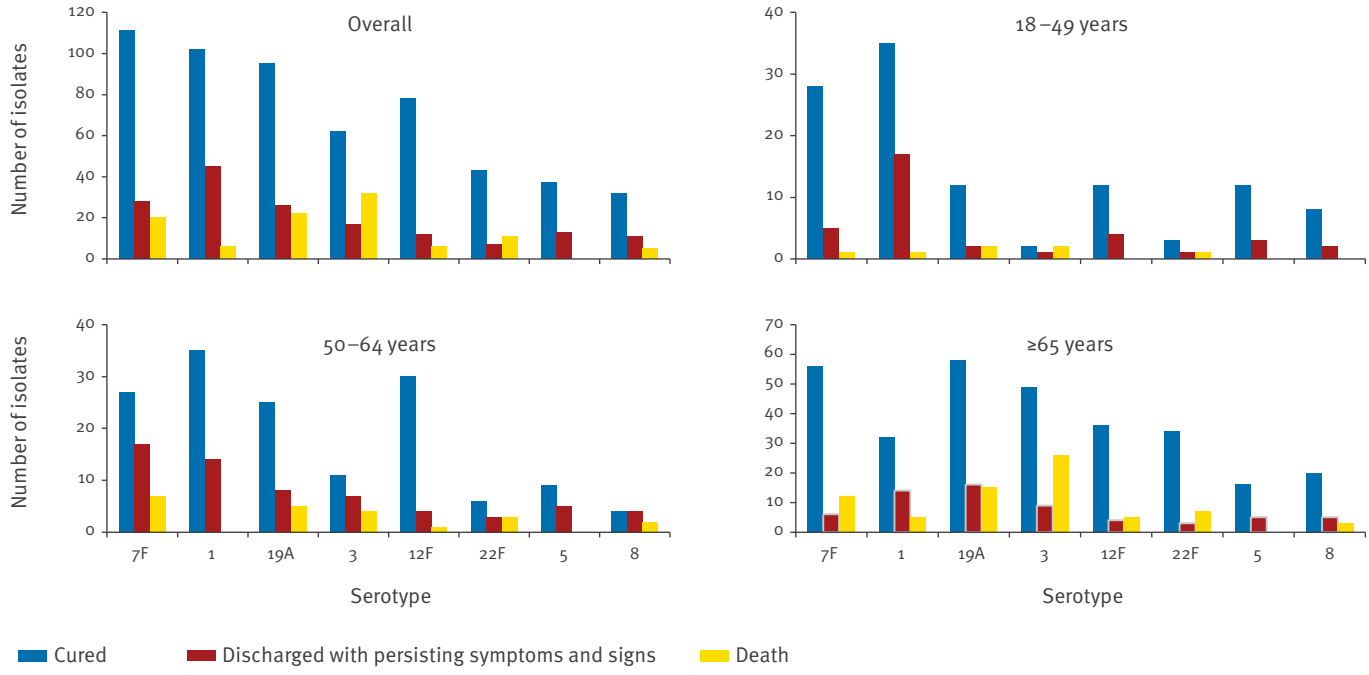
Category	n	Outcome at discharge			Univariate OLR		Multivariate OLR	
		Cured n (%)	Discharged with persisting symptoms n (%)	Death n (%)	Odds ratio (95% CI)	P value	Odds ratio (95% CI)	P value
Total[a]	1,329	884 (67)	237 (18)	208 (16)	–	–	–	–
Comorbidity								
No	313	217 (69)	67 (21)	29 (9)	1	–	–	–
Any	1,016	667 (66)	170 (17)	179 (18)	1.29 (0.98–1.69)	0.067	1.20 (0.91–1.60)	0.20
Number of comorbidities, mean ± SD	1,323	1.43 ± 1.19	1.4 ± 1.22	1.88 ± 1.28	1.18 (1.08–1.29)	0.0004	–	–
COPD	335	226 (68)	56 (17)	53 (16)	0.97 (0.75–1.26)	0.83	0.90 (0.69–1.19)	0.47
Asthma	68	47 (69)	12 (18)	9 (13)	0.88 (0.52–1.48)	0.63	0.88 (0.51–1.51)	0.65
Heart failure	249	141 (57)	44 (18)	64 (26)	1.85 (1.41–2.43)	<0.0001	1.70 (1.26–2.30)	0.0006
Renal insufficiency	179	103 (58)	25 (14)	51 (29)	1.82 (1.34–2.48)	0.0001	1.63 (1.16–2.29)	0.0047
Hepatic disease	104	62 (60)	15 (14)	27 (26)	1.56 (1.05–2.31)	0.027	1.38 (0.91–2.10)	0.13
Immunosuppression	172	116 (67)	28 (16)	28 (16)	0.99 (0.71–1.38)	0.93	0.96 (0.67–1.39)	0.84
HIV infection	24	18 (75)	3 (13)	3 (13)	0.68 (0.27–1.70)	0.41	0.96 (0.37–2.50)	0.93
Cancer	296	192 (65)	48 (16)	56 (19)	1.17 (0.90–1.52)	0.26	1.16 (0.87–1.54)	0.31
Diabetes	203	136 (67)	37 (18)	30 (15)	0.98 (0.72–1.34)	0.91	0.83 (0.59–1.15)	0.26
Asplenia	11	6 (55)	1 (9)	4 (36)	2.15 (0.70–6.55)	0.18	2.18 (0.69–6.87)	0.18
Alcoholism	59	28 (48)	13 (22)	18 (31)	2.37 (1.45–3.86)	0.0006	2.79 (1.65–4.73)	0.0001
Hypertension	78	54 (69)	12 (15)	12 (15)	0.89 (0.55–1.45)	0.65	0.92 (0.56–1.52)	0.74
Smoking	26	21 (81)	4 (15)	1 (4)	0.44 (0.16–1.20)	0.11	0.59 (0.13–1.62)	0.30
Previous IPD	14	7 (50)	4 (29)	3 (21)	1.82 (0.67–4.97)	0.24	1.84 (0.65–5.19)	0.25
Tuberculosis	8	5 (63)	0 (0)	3 (38)	1.68 (0.44–6.37)	0.45	2.04 (0.52–7.91)	0.31
Other	154	101 (66)	29 (19)	24 (16)	1.05 (0.74–1.48)	0.79	1.05 (0.73–1.51)	0.78

CI: confidence interval; COPD: chronic obstructive pulmonary disease; HIV: human immunodeficiency virus; IPD: invasive pneumococcal disease; OLR: ordinal logistic regression; SD: standard deviation.

[a] Data missing for three patients.

Figure 1

Disease outcome at hospital discharge by age and serotype for patients with invasive pneumococcal disease, Belgium, 2009–2011 (n=1,214)



Only the serotypes accounting for at least 4% of isolates overall are shown.

bacteraemic pneumonia (13% (128/968)) and bacteraemia without focus (17% (11/64)), serotype 1 in those with empyema (23% (18/80)), and serotype 19A in cases of meningitis (11% (7/65)).

Serotypes 12F and 22F, not included in PCV13, accounted for 96 (8%) and 61 (5%) of the 1,214 IPD serotyped isolates, respectively. Serotypes 3 (17% (32/188)), 19A (12% (22/188)), and 7F (11% (20/188)) together accounted for nearly 40% of the 188 deaths with available serotype data. The highest case fatality rate was for serotype 6B (6 deaths for 11 cases; 55%) but eight of the 11 patients were ≥65 years of age. Among the six most frequent serotypes, the case fatality rates were 13% (20/159) for serotype 7F, 4% (6/153) for serotype 1, 15% (22/143) for serotype 19A, 29% (32/111) for serotype 3, 6% (6/96) for serotype 12F, and 18% (11/61) for serotype 22F. None of the 50 patients with serotype 5 IPD died.

Most pneumococcal isolates were susceptible to the four antibiotics tested (Table 5). Among the 22 pneumococcal isolates non-susceptible to penicillin, 18 were of serotype 19A. Overall, among the 145 serotype 19A isolates, 14 (10%) were intermediately resistant (MIC= 4 mg/L) and four (3%) resistant (MIC ≥8 mg/L) to penicillin, 95 (66%) were resistant to tetracycline, and 85 (59%) were resistant to erythromycin. Serotype 1, represented by a total of 154 isolates, was also frequently resistant to tetracycline (76 isolates (49%)) and erythromycin (74 isolates (48%)). The 159 serotype 7F isolates were all susceptible to the four antibiotics tested.

Overall, infections caused by the seven serotypes included in PCV7 (serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F) represented 7% (83/1,214) of IPD cases (Figure 2). Also, 54% (659/1,214) of cases were caused by the six additional serotypes included in PCV13 (serotypes 1, 3, 5, 7F, 19A, and 6A), while 25% (298/1,214) were caused by the additional serotypes included in PPV23, and 14% (174/1,214) were caused by serotypes not included in any of the pneumococcal vaccines. Among the patients ≥50 years of age, the proportion of PCV7 serotypes decreased from 10% (33/325) in 2009 to 5% (13/252) in 2011 (p=0.028).

Discussion

In 2009, a prospective, active hospital-based study of morbidity and mortality of IPD in adults was started in Belgium. Data collected between 2009 and 2011 showed that mortality due to IPD was high, with up to 20% case fatality in adults ≥65 years of age. Bacteraemic pneumonia was the most frequent clinical type of IPD. Of the 1,214 serotyped isolates, 742 (61%) were included in PCV13, which thus included the most resistant and lethal isolates.

As in the current study, previous studies in the Netherlands, Spain and the United States (US) have shown that bacteraemic pneumonia predominates in adults [9-11]. Meningitis has been reported to be more frequent in young children [11,12]. In addition, we

Table 5

Antibiotic susceptibility of pneumococcal isolates derived from patients with invasive pneumococcal disease, Belgium, 2009–2011 (n=1,214)

Antibiotic	Pneumococcal isolates, n (%)				Resistant serotypes n (%)[a]
	Susceptible	Intermediate	Resistant	Unknown	
Penicillin	1,189 (98)	14 (1)	8 (1)	3 (<1)	19A: 4 (3); 14: 1 (4); 35: 1 (7); 15A: 1 (8); 20: 1 (20)
Tetracycline	945 (78)	2 (<1)	264 (22)	3 (<1)	1: 76 (49); **19A: 95 (66)**; 12F: 27 (28); 3: 1 (1); 22F: 1 (2); 5: 2 (4); 8: 2 (4); 6A: 8 (20); 14: 9 (39); 33F: 2 (9); 11A: 1 (5); 24F: 5 (26); 35: 1 (7); **15A: 6 (50); 6B: 6 (55)**; 38: 1 (10); 19F: 2 (20); 15F: 2 (29); 9V: 2 (33); 23A: 2 (33); 9A: 1 (20); 20: 1 (20); **15B: 2 (50)**; 15C: 1 (25); **9: 2 (67)**; **15: 1 (50)**; **12B: 2 (50)**; **13: 1 (100)**
Erythromycin	954 (79)	2 (<1)	255 (21)	3 (<1)	1: 74 (48); **19A: 85 (59)**; **14: 13 (57)**; **33F: 14 (64)**; 3: 1 (1); 12F: 5 (5); 22F: 1 (2); 8: 1 (2); 6A: 9 (23); 11A: 4 (19); 9N: 1 (5); 24F: 6 (32); 35: 1 (7); 23B: 1 (8); **15A: 7 (58)**; **6B: 7 (64)**; 38: 1 (10); **19F: 9 (70)**; 15F: 1 (14); 9V: 2 (33); 23A: 2 (33); 9A: 1 (20); 20: 1 (20); 15B: 1 (25); 15C: 1 (25); **33: 1 (100)**
Ofloxacin	1,206 (99)	0 (0)	3 (<1)	5 (<1)	1: 1 (1); 14: 1 (4); 29: 1 (14)

[a] Percentages in this column relate to the proportion of resistant isolates per total isolates of a given serotype. Serotypes with more than 50% of isolates resistant are shown in bold.

confirmed age as a risk factor for IPD and death due to IPD [11]. Chronic illness is another well-known risk factor for IPD [13-15]. In our study, more than three-quarters of patients with IPD had at least one chronic underlying condition. This proportion was even higher for older adults. This confirms that patients with comorbidities have a higher risk of developing IPD. Patients with at least one comorbidity generally also had a higher risk of death in hospital due to IPD.

Universal mass vaccination of children aged <2 years with PCV7 has dramatically decreased the incidence of vaccine-type IPD in this population and, to a lesser extent, in older individuals through herd effect [16-18]. Nevertheless, even with successful mass vaccination, IPD remains a problem. The ongoing national surveillance will help determine how routine use of PCV13 in children further influences the epidemiology of IPD in adults. While PCV7-type IPD has decreased [17,18], non-PCV7-type IPD has risen in many countries [17,19-23]. Accordingly, we found 7% of IPD cases in adults caused by serotypes included in PCV7. Because four of the most frequent serotypes (7F, 1, 19A, and 3) in our study are included in the newly licensed PCV13, they should become less common as the use of PCV13 increases.

Our finding that serotypes 1, 7F, and 19A predominate corresponds with other reports [6,19,23-25]. Serotype 19A was the third most prevalent serotype in adult IPD. Similarly, this serotype was previously reported as the second or third most prevalent serotype in IPD in children <5 years of age [6,7]. This is a concern because serotype 19A is frequently multi-resistant to antibiotics. The incidence of serotype 19A started to increase in children before the introduction of PCV7 and further increased after its introduction [6], suggesting that the rise is partly due to other factors, such as antibiotic consumption and secular trends. Serotypes 12F and 22F were the fifth and sixth most common serotype. Serotype 12 F has also become more common in young children since 2002–2003 [6]. Both serotypes are included in PPV23 but not in PCV13 and should be closely monitored in the future.

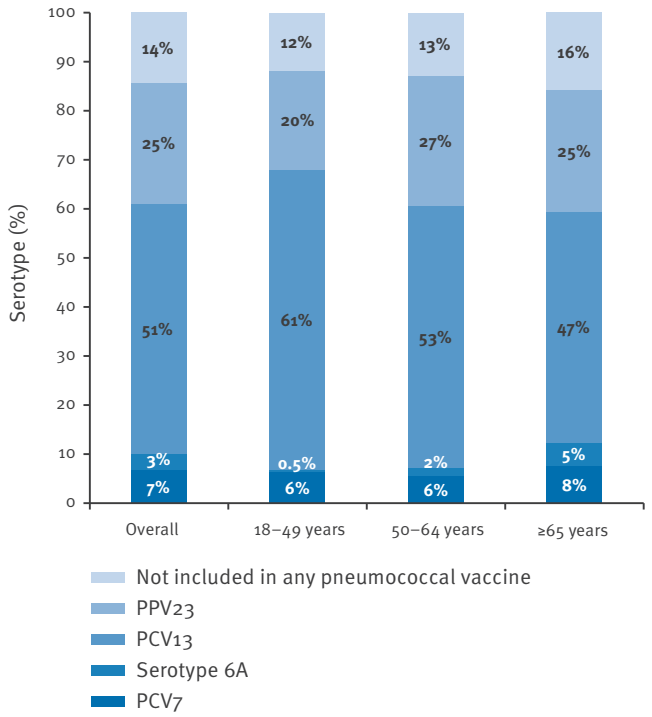
According to a review article, the reported case fatality rate for adult patients hospitalised with IPD has remained relatively stable at approximately 12% since the 1950s [26]. We found a slightly higher rate of 16%. The case fatality rate was low for serotypes 1 and 5 and high for serotypes 3 and 6B, as shown in previous studies in Denmark, the Netherlands and the US, [16,27,28].

One limitation of our study is that older patients may have been over-represented because adults between 18 and 49 years of age were included only from the second year of study (2010), whereas adults≥50 years of age were included from the beginning of the study (2009). However, this should have little impact on the results because per year the majority (51%) of patients were ≥65 years of age, and the data were analysed per age group. Another possible bias of the results is that 543 of the 1,875 (28%) eligible patients were not included in the analysis due to unavailable informed consent or late blood draw. Disease in these patients may have been more severe (patients in ICU) or less severe (patients who left the hospital before microbiological confirmation of IPD) than in the analysed population.

In conclusion, this study showed that, in Belgium, the mortality of IPD in adults is high, with a case fatality rate of 20% in patients ≥65 years of age. The most common and virulent pneumococcal serotypes are included in PCV13, which adds support for the use of

Figure 2

Proportion of invasive pneumococcal disease caused by serotypes included in pneumococcal vaccines, Belgium, 2009–2011 (n=1,214)



The 7-valent pneumococcal conjugate vaccine (PCV7) included serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F. The 13-valent pneumococcal conjugate vaccine (PCV13) included additional serotypes 1, 3, 5, 6A, 7F, and 19A. Compared to PCV13, the 23-valent pneumococcal polysaccharide vaccine (PPV23) did not comprise serotype 6A, but included additional serotypes 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20, 22F, and 33F. Serotype 6A was presented separately because it is included in PCV13 but not in PPV23. The total may be different from 100% due to rounding.

this vaccine in combination with the PPV23 in high-risk and older adults. In addition, these data are essential when assessing the impact of PCV13 vaccination in adults in the future.

Acknowledgements

Acknowledgement: The authors thank all the investigators who have contributed to the surveillance. The authors also thank Patricia Slachmuylders and Annick Mignon (Pfizer, Belgium) for study coordination, Pr. Adelin Albert and Nadia Dardenne (Université de Liège, Belgium) for statistical analysis, and Dr. Julie Harriague (4Clinics, France) for writing assistance.

Funding: This study was supported by Pfizer Belgium. Support for the writing assistance was also provided by Pfizer Belgium.

Conflict of interest

Jan Verhaegen has received grants from Pfizer. J. F. has received personal fees from Pfizer and is an advisor for the vaccine workgroup of the High Council of Public Health of the Belgian Federal Government and for the vaccine workgroup of the Flemish Government (unpaid activities). B. D. has received personal fees from Pfizer. K. V. H. has received consulting fees from Pfizer, research grants from Pfizer and GlaxoSmithKline Biologicals, and speaker fees from several vaccine manufacturers. Y. V. L. received personal and travel fees from Pfizer. P. V. D. acts as chief and principal investigator for vaccine trials conducted on behalf of the University of Antwerp, for which the University has obtained research grants from vaccine manufacturers. W. P. has received funds for advisory board membership from Pfizer, Bayer, AstraZeneca, GlaxoSmithKline Biologicals, Merck-Shering-Plough, and Astellas and research grants from Pfizer, Sanofi-Aventis, Bayer, and AstraZeneca. F. S. was an employee of Pfizer, which has a licensed pneumococcal conjugate vaccine, at the time of the study conduct. W. D. B.: none to declare. Writing assistance in preparation of this manuscript was provided by Dr. Julie Harriague (4 Clinics, Paris, France). This assistance included preparation of the first draft, incorporation of authors' contributions and revisions, and editing, all under the direction of the authors. At all stages, the authors had control over the content of this manuscript, for which they gave final approval and take full responsibility.

Authors' contributions

All authors participated actively since 2009 on the set-up of the study protocol, the follow-up of the study and the preparation of the manuscript.

References

1. Centers for Disease Control and Prevention. Update: pneumococcal polysaccharide vaccine usage--United States. MMWR Morb Mortal Wkly Rep. 1984;33(20):273-6, 281.
2. Conseil Supérieur de la Santé. Vaccination antipneumococcique de l'adulte (2013). [Pneumococcal vaccination for adults]. Avis CSS n° 8817. Brussels: Conseil Supérieur de la Santé; 2013. French. Available from: http://www.health.belgium.be/eportal/Aboutus/relatedinstitutions/SuperiorHealthCouncil/publications/factsheetsvaccination/index.htm#.UuorQD2E2Rc
3. Conseil Supérieur de la Santé. Recommandations pour la vaccination des enfants présentant un risque accru de maladie invasive à pneumocoques (MIP). [Vaccine recommendations for children at increased risk of invasive pneumococcal disease]. Avis CSS n° 8757. Brussels: Conseil Supérieur de la Santé; 2013. French. Available from: http://www.health.belgium.be/eportal/Aboutus/relatedinstitutions/SuperiorHealthCouncil/publications/factsheetsvaccination/index.htm#.UuorQD2E2Rc
4. European Medicines Agency. Prevenar 13 (pneumococcal polysaccharide conjugate vaccine, 13-valent adsorbed) CMHP variation assessment report. London: European Medicines Agency; 2011. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Assessment_Report_-_Variation/human/001104/WC500119784.pdf
5. Institut Scientifique de Santé Publique (WIV-ISP). 2011 Annual report on pneumococcal surveillance. Brussels: WIV-ISP; 2011. Available from: https://www.wiv-isp.be/epidemio/epifr/plabfr/plabanfr/11_030f_r.pdf
6. Hanquet G, Lernout T, Vergison A, Verhaegen J, Kissling E, Tuerlinckx D, et al. Impact of conjugate 7-valent vaccination in Belgium: Addressing methodological challenges. Vaccine. 2011;29(16):2856-64. http://dx.doi.org/10.1016/j.vaccine.2011.02.016
7. Vergison A, Tuerlinckx D, Verhaegen J, Malfroot A. Epidemiologic features of invasive pneumococcal disease in Belgian children: passive surveillance is not enough. Pediatrics. 2006;118(3):e801-9. http://dx.doi.org/10.1542/peds.2005-3195
8. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing: Twentieth Informational Supplement. Wayne, PA: CLSI; 2012. CSLI document M100-S22.
9. Ardanuy C, Marimon JM, Calatayud L, Gimenez M, Alonso M, Grau I, et al. Epidemiology of invasive pneumococcal disease in older people in Spain (2007-2009): implications for future

vaccination strategies. PLoS One. 2012;7(8):e43619. http://dx.doi.org/10.1371/journal.pone.0043619

10. Bliss SJ, Larzalere-Hinton F, Lacapa R, Eagle KR, Frizzell F, Parkinson A, et al. Invasive pneumococcal disease among White Mountain Apache adults, 1991-2005. Arch Intern Med. 2008;168(7):749-55. http://dx.doi.org/10.1001/archinte.168.7.749
11. Jansen AG, Rodenburg GD, de Greeff SC, Hak E, Veenhoven RH, Spanjaard L, et al. Invasive pneumococcal disease in the Netherlands: Syndromes, outcome and potential vaccine benefits. Vaccine. 2009;27(17):2394-401. http://dx.doi.org/10.1016/j.vaccine.2009.01.127
12. Institut Scientifique de Santé Publique (WIV-ISP). Surveillance des maladies infectieuses chez les enfants en Belgique. PediSurv – Rapport annuel 2010 [Surveillance of infectios diseases in children in Belgium – 2010 annual report]. Brussels: WIV-ISP; 2010. Available from: https://www.wiv-isp.be/PEDISURV/AnnualReports/2010/jaarverslag_2010_fr.pdf
13. Prevention of pneumococcal disease: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep. 1997;46(RR-8):1-24.
14. Kyaw MH, Rose CE Jr, Fry AM, Singleton JA, Moore Z, Zell ER, et al. The influence of chronic illnesses on the incidence of invasive pneumococcal disease in adults. J Infect Dis. 2005;192(3):377-86. http://dx.doi.org/10.1086/431521
15. van Hoek AJ, Andrews N, Waight PA, Stowe J, Gates P, George R, et al. The effect of underlying clinical conditions on the risk of developing invasive pneumococcal disease in England. J Infect. 2012;65(1):17-24. http://dx.doi.org/10.1016/j.jinf.2012.02.017
16. Lexau CA, Lynfield R, Danila R, Pilishvili T, Facklam R, Farley MM, et al. Changing epidemiology of invasive pneumococcal disease among older adults in the era of pediatric pneumococcal conjugate vaccine. JAMA. 2005;294(16):2043-51. http://dx.doi.org/10.1001/jama.294.16.2043
17. Pilishvili T, Lexau C, Farley MM, Hadler J, Harrison LH, Bennett NM, et al. Sustained reductions in invasive pneumococcal disease in the era of conjugate vaccine. J Infect Dis. 2010;201(1):32-41. http://dx.doi.org/10.1086/648593
18. Whitney CG, Farley MM, Hadler J, Harrison LH, Bennett NM, Lynfield R, et al. Decline in invasive pneumococcal disease after the introduction of protein-polysaccharide conjugate vaccine. N Engl J Med. 2003;348(18):1737-46. http://dx.doi.org/10.1056/NEJMoa022823
19. Aguiar SI, Brito MJ, Goncalo-Marques J, Melo-Cristino J, Ramirez M. Serotypes 1, 7F and 19A became the leading causes of pediatric invasive pneumococcal infections in Portugal after 7 years of heptavalent conjugate vaccine use. Vaccine. 2010;28(32):5167-73. http://dx.doi.org/10.1016/j.vaccine.2010.06.008
20. Hicks LA, Harrison LH, Flannery B, Hadler JL, Schaffner W, Craig AS, et al. Incidence of pneumococcal disease due to non-pneumococcal conjugate vaccine (PCV7) serotypes in the United States during the era of widespread PCV7 vaccination, 1998-2004. J Infect Dis. 2007;196(9):1346-54. http://dx.doi.org/10.1086/521626
21. Miller E, Andrews NJ, Waight PA, Slack MP, George RC. Herd immunity and serotype replacement 4 years after seven-valent pneumococcal conjugate vaccination in England and Wales: an observational cohort study. Lancet Infect Dis. 2011;11(10):760-8. http://dx.doi.org/10.1016/S1473-3099(11)70090-1
22. Mu-oz-Almagro C, Jordan I, Gene A, Latorre C, Garcia-Garcia JJ, Pallares R. Emergence of invasive pneumococcal disease caused by nonvaccine serotypes in the era of 7-valent conjugate vaccine. Clin Infect Dis. 2008;46(2):174-82. http://dx.doi.org/10.1086/524660
23. Singleton RJ, Hennessy TW, Bulkow LR, Hammitt LL, Zulz T, Hurlburt DA, et al. Invasive pneumococcal disease caused by nonvaccine serotypes among alaska native children with high levels of 7-valent pneumococcal conjugate vaccine coverage. JAMA. 2007;297(16):1784-92. http://dx.doi.org/10.1001/jama.297.16.1784
24. Grall N, Hurmic O, Al Nakib M, Longo M, Poyart C, Ploy MC, et al. Epidemiology of Streptococcus pneumoniae in France before introduction of the PCV-13 vaccine. Eur J Clin Microbiol Infect Dis. 2011;30(12):1511-9. http://dx.doi.org/10.1007/s10096-011-1251-9
25. Regev-Yochay G, Rahav G, Strahilevitz J, Bishara J, Katzir M, Chowers M, et al. A nationwide surveillance of invasive pneumococcal disease in adults in Israel before an expected effect of PCV7. Vaccine. 2013;31(19):2387-94. http://dx.doi.org/10.1016/j.vaccine.2013.02.059
26. Ludwig E, Bonanni P, Rohde G, Sayiner A, Torres A. The remaining challenges of pneumococcal disease in adults. Eur Respir Rev. 2012;21(123):57-65. http://dx.doi.org/10.1183/09059180.00008911
27. Jansen AG, Rodenburg GD, van der Ende A, van Alphen L, Veenhoven RH, Spanjaard L, et al. Invasive pneumococcal disease among adults: associations among serotypes, disease characteristics, and outcome. Clin Infect Dis. 2009;49(2):e23-9. http://dx.doi.org/10.1086/600045
28. Martens P, Worm SW, Lundgren B, Konradsen HB, Benfield T. Serotype-specific mortality from invasive Streptococcus pneumoniae disease revisited. BMC Infect Dis. 2004;4:21. http://dx.doi.org/10.1186/1471-2334-4-21

Information resources and latest news about Ebola virus disease available from ECDC

Eurosurveillance editorial team (eurosurveillance@ecdc.europa.eu)[1]
1. European Centre for Disease Prevention and Control (ECDC) Stockholm, Sweden

Citation style for this article:
Eurosurveillance editorial team. Information resources and latest news about Ebola virus disease available from ECDC. Euro Surveill. 2014;19(31):pii=20871. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20871

Article published on 07 August 2014

The European Centre for Disease Prevention and Control (ECDC) has a health topic page dedicated to providing comprehensive information about the Ebola virus disease (EVD) outbreak in West Africa [1]. The page links to public health advice and the ECDC risk assessment on EVD. The Centre has published information to travellers, available in 22 European Union languages, as well as epidemiological updates.

The EVD outbreak is evolving in West Africa since December 2013. The outbreak is centred to Guinea, Liberia and Sierra Leone and intensified during June and July 2014. A total of 108 new cases of EVD (laboratory-confirmed, probable and suspect) and 45 deaths were reported from Guinea, Liberia, Sierra Leone and Nigeria between 2 and 4 August 2014, bringing the total number of cases to 1,711 with 932 deaths [2].

The European Centre for Disease Prevention and Control is monitoring the development of the outbreak closely and evaluates the risk of importation of the disease to the EU, the risk of spreading, as well as the risk to EU travellers and residents in the affected areas in West Africa continuously.

References

1. European Centre for Disease Prevention and Control (ECDC). Stockholm: ECDC; August 2014. Available from: http://www.ecdc.europa.eu/en/healthtopics/ebola_marburg_fevers/pages/index.aspx
2. World Health Organization (WHO). Ebola virus disease update - West Africa. Geneva: WHO; 2014. Available from: http://www.who.int/csr/don/2014_08_06_ebola/en/

European Commission launches consultation on Science 2.0

Eurosurveillance editorial team (eurosurveillance@ecdc.europa.eu)[1]
1. European Centre for Disease Prevention and Control (ECDC) Stockholm, Sweden

Citation style for this article:
Eurosurveillance editorial team. European Commission launches consultation on Science 2.0. Euro Surveill. 2014;19(31):pii=20872. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20872

Article published on 07 August 2014

The European Commission has launched a public consultation on 'Science 2.0' which is open until 30 September 2014 [1]. The consultation has three main objectives: (i) to assess the degree of awareness among stakeholders, (ii) assess the perception of opportunities and challenges, and (iii) to identify policy implications and actions to strengthen the competitiveness of the European science and research system [2]. A suggested new approach to science, 'Science 2.0' uses information-sharing and collaboration made possible by network technologies [3].

The 'Science 2.0' consultation paper stresses for example that open access needs to be considered in a broader context and that the dynamics of 'Science 2.0' will further expand open access requirements [2]. The European Commission has made open access to peer-reviewed publications the default position across Horizon 2020, the biggest research and innovation programme ever adopted by the EU [4]. With EUR 80 billion funding from 2014 to 2020, it is the financial instrument used to implement 'Innovation Union', a cornerstone initiative of Europe 2020, the EU growth strategy which aims to secure the global competitiveness of Europe.

An objective of the European Union (EU) is to strengthen its scientific and technological bases by achieving a European Research Area where researchers, knowledge and technology circulate freely [2]. As part of this, most EU Member States have established legal conditions to support e.g. open access to publications

References

1. European Commission. Consultation on 'Science 2.0': Science in Transition. [Accessed 7 Aug 2014]. Available from: http://ec.europa.eu/research/consultations/science-2.0/consultation_en.htm
2. European Commission. Background document. Public consultation. 'Science 2.0': Science in Transition. [Accessed 7 Aug 2014]. Available from: http://ec.europa.eu/research/consultations/science-2.0/background.pdf
3. Wikipedia. [Accessed 7 Aug 2014]. Available from: http://en.wikipedia.org/wiki/Science_2.0
4. European Union. What is Horizon 2020 ? . [Accessed 7 Aug 2014]. Available from: http://ec.europa.eu/programmes/horizon2020/en/what-horizon-2020