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Widespread Usutu virus outbreak in birds in the Netherlands, 2016

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We report a widespread Usutu virus outbreak in birds in the Netherlands. Viral presence had been detected through targeted surveillance as early as April 2016 and increased mortality in common blackbirds and captive great grey owls was noticed from August 2016 onwards. Usutu virus infection was confirmed by post-mortem examination and RT-PCR. Extensive Usutu virus activity in the Netherlands in 2016 underlines the need to monitor mosquito activity and mosquito-borne infections in 2017 and beyond.

Here we describe the detection of Usutu virus (USUV; genus *Flavivirus*, family *Flaviridae*), a potentially zoonotic mosquito-borne virus, in live birds captured in the Netherlands in April 2016, and the development of an USUV outbreak with mortality in birds first noticed in August 2016. We provide details on pathological findings in common blackbirds (*Turdus merula*; Tm) and great grey owls (*Strix nebulosa*; Sn) and give information on the size of the outbreak, as well as on mosquito abundance in 2016.

Subclinical bird cases

As part of a targeted study looking at potential routes of incursion of arboviruses, live birds have been captured for sample collection since March 2016. USUV RNA was detected in throat swabs from two healthy blackbirds caught near Wageningen (Gelderland Province) in early April, based on RT-PCR detection of two independent USUV genome targets and sequencing of a 214 bp genome fragment generated in a third, pan-flavi RT-PCR [1,2].

Outbreak in birds

Outbreak identification (first set of birds)

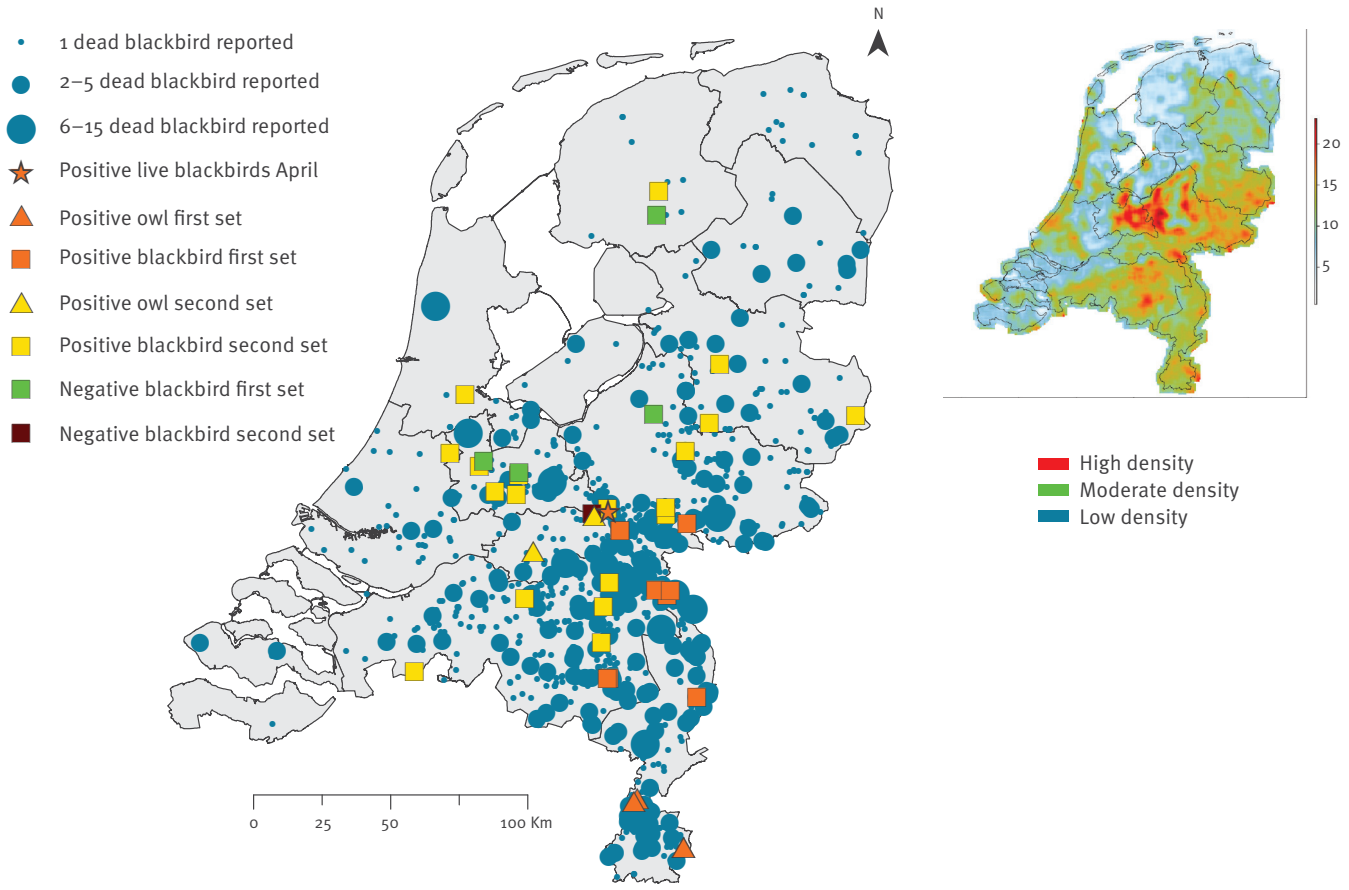
The first evidence for an outbreak was obtained in the period from 28 August to 13 September 2016, when an increasing number of case reports of disease-associated mortality in blackbirds were put forward through a citizen science-based alerting system (Table 1). In parallel, the number of blackbirds submitted for post-mortem examination in the context of wildlife disease scanning increased. Eighteen blackbirds were submitted in 2016 until 13 September, and among these one (Tm 1) was obtained on 10 August 2016 and 12 (Tm 2–12, plus one autolytic specimen) were obtained from 28 August onwards (Table 1). Tm 1–12 were from 11 different sites.

During the same period, the deaths of four captive great grey owls (Sn 1–4) were investigated. The deaths occurred between 13 August and 12 September 2016, in three facilities. The post-mortem findings in birds Tm 1–12 and Sn 1–4 are summarised in Table 2.

Initially, based on the presence of *Plasmodium* spp. schizonts and mixed inflammatory infiltrates in mainly liver and spleen, avian malaria was diagnosed (Tm 1–3, 5) [3,4]. However, when birds had myocardial degeneration (Tm 4) or encephalitis (Tm 7–8, Sn 4), tissues were submitted for USUV RT-PCR. USUV was detected in eight of 12 blackbirds (Tm 4, 6–12) and all four great grey owls (Table 2). USUV-positive cases came from sites located in the south-east of the Netherlands (Figure 1, first set). Public health authorities were

Figure 1

Spatial distribution of the common blackbird and great grey owl specimens tested for Usutu virus infection and common blackbird mortality as reported by the public, the Netherlands, 1 August–23 September 2016 (inset: common blackbird density 2013–15)



First set: the birds examined post mortem from 1 August to 13 September 2016; second set: those obtained from 14 to 23 September 2016.

informed of the outbreak, followed by a press release to inform the public on 15 September 2016.

Scale of the outbreak (second set of birds)

To gain insight in the spatial distribution of the USUV outbreak, more information was collected on deaths among blackbirds and great grey owls outside the initially identified area of USUV activity (south-east of the Netherlands). The number of reported dead blackbirds per location was extracted from reports by the public to Sovon or the Dutch Wildlife Health Centre from 1 August to 23 September 2016 and mapped using ArcGIS software by Esri (Figure 1). To visually compare this with the blackbird population density, a species distribution model was made based on more than 10,000 standardised five-minute bird counts performed during the breeding seasons from 2013 to 2015, according to a fixed grid and a large set of explanatory variables [5] (Figure 1 inset). A selection of dead blackbirds and great grey owls notified for submission by the public or owl owners between 14 to 23 September were collected for USUV testing. The selection was based on how fresh the carcass was and whether it was found at a location where USUV activity had not been identified before.

There were 924 citizen reports of which 226 mentioned that multiple sick or dead blackbirds had been observed. Most reports were from September (885/924, 96%) and from the provinces Noord Brabant (293/924, 32%), Gelderland (261/924, 28%) and Limburg (148/924, 16%). Between 14 and 23 September, 20 dead blackbirds and two great grey owls were collected for USUV testing. Nineteen of the blackbirds and two of the great grey owls tested positive for USUV (Figure 1, second set). These data support widespread occurrence of USUV infection in birds in the Netherlands in September 2016.

Vector abundance

Long-term standardised datasets on mosquito abundance are not available in the Netherlands, and arbovirus surveillance in mosquitoes is not performed. An indication of mosquito abundance in 2016 relative to previous years was obtained from data on mosquitoes found at four locations, with bi-weekly collection

Table 1

Common blackbirds (Turdus merula) observed by citizens to die of disease (n = 136) and those submitted for post-mortem examination (n = 115), the Netherlands, 2005–16

Time period	Proportion of dead blackbirds reported to Sovon[a] with 'disease' as the cause of death		Dead blackbirds investigated at DWHC[b]
	Disease/total deaths	%	Number
2005	0/11	0	NA
2006	0/367	0	NA
2007	0/232	0	NA
2008	1/160	1	0
2009	109/473[c]	23	4
2010	1/161	1	12
2011	0/111	0	3
2012	13/388	3	49[d]
2013	1/103	1	18
2014	2/102	2	5
2015	0/120	0	6
2016 until 13 Sep	9/95[e]	9	18[e]

NA: not available.

[a] Dutch Centre for Field Ornithology, Nijmegen.

[b] Dutch Wildlife Health Centre, Utrecht (operational in Utrecht from 2008 onwards).

[c] All reports indicating blackbirds that died of disease were preceded by the press paying attention to the *Trichomonas gallinae* finch epidemic.

[d] Fourty-seven of the blackbirds were obtained following the reports in the national press on Usutu virus infection in Germany and a press release on 7 October requesting the public to submit dead blackbirds. There was no evidence for Usutu virus infection at the time [1].

[e] Among these, eight of nine diseased birds reported to Sovon and 12 of 18 submissions to DWHC were obtained during the 16-day window from 28 August to 13 September. These increasing numbers were not triggered by media attention.

of mosquitoes carried out during the summer period in the years 2014 to 2016 using one trap design (BG-sentinel trap, Biogents, Germany) at sites where no insecticide treatment was applied. The total number trapped across sites in 2016 (n = 25,693) was approximately six times greater than in 2014 (n = 4,558) and approximately 10 times greater than in 2015 (n = 2,615) (Figure 2). None of the mosquito samples were tested for USUV.

Discussion

There is a widespread USUV outbreak in wild blackbirds and captive great grey owls in the Netherlands. Although USUV circulated in neighbouring countries, it had not been detected in the Netherlands before 2016, despite scanning surveillance for bird mortality since 2008 and a targeted study in dead blackbirds based on convenience sampling in 2012 [1]. USUV emerged in Europe in Italy 20 years ago [6]; however, introductions from Africa probably started several decades earlier and continue to occur [7]. The virus has been detected in mosquitoes, birds and bats in eight European countries (Austria, Belgium, Czech Republic, Germany, Hungary, Italy, Spain, Switzerland) [7,8] and is presumably maintained in enzootic mosquito–bird transmission cycles. Birds of 14 orders can be infected [8]. In the current outbreak in the Netherlands, live bird monitoring showed the presence of the virus in wild birds already months before the detection of unusual death rates among blackbirds and great grey owls. USUV outbreaks also occurred in birds in neighbouring countries, Belgium and Germany, in 2016 (personal communication: M. Garigliany and J. Schmidt-Chanasit, August 2016). A comprehensive genetic study including strain data from affected neighbouring countries is underway to elucidate the origin of events and patterns of spread.

High mosquito abundance may have been one of the factors contributing to the occurrence and scale of the outbreak in the Netherlands. In Europe, the *Culex pipiens* mosquito is considered an important vector for USUV [9,10]. The *Culex pipiens/torrentium* complex is found throughout the Netherlands between April and October [11]. June 2016 was extremely wet and, together with unusually high temperatures in September, may have furthered and prolonged mosquito activity [12,13]. The event demonstrates the need for long-term standardised datasets on mosquito abundance in the Netherlands and their analysis in relation to climate. The samples of captured mosquitoes could be one pillar in a molecular surveillance programme for USUV and other mosquito-borne zoonotic viruses.

In birds, fatal infections occur mostly in *Passeriformes* and *Strigiformes* [9,14-17]. Hepatosplenomegaly is a common finding. Histological lesions include encephalitis and necrosis in heart, liver, spleen and kidney,

Table 2

Pathological findings in the common blackbirds (Tm 1–12) and great grey owls (Sn 1–4) submitted, grouped by detected infectious agent(s), the Netherlands, 1 August–13 September

	Blackbird			Owl	
	Tm 1–3,5	Tm 4,7,8,11,12	Tm 6,9,10	Sn 1–3	Sn 4
Infectious agent(s) detected[a]	Only Plasmodium	Plasmodium and USUV	Only USUV	Plasmodium and USUV	Only USUV
Gross lesions [b,c]					
Hepatomegaly	4/4	3/5	1/3	2/3	1/1
Splenomegaly	4/4	4/5	3/3	3/3	1/1
Lung hyperaemia, oedema	2/4	3/5	1/3	2/3	1/1
Heart abnormalities	2/4 (1 haemopericardium, 1 pale)	1/5 (1 pale)	0/3	1/3 (1 hydropericardium)	0/1
Skin by cloaca firm, crusty	2/4	5/5	1/3	0/3	0/1
Feather abnormalities	0/4	2/5 (1 rfsh, 1 blood pens)	2/3 (2 featherless heads)	0/3	0/1
Histological lesions [b,c]					
Encephalitis	0/3	2/4 (1 pvc, 1 gli/deg/pvc/swe)	1/3 (1 pvc)	0/3	1/1 (1 mix/gli)
Myocardial degeneration	0/4	1/5	1/3	0/3	1/1
Myocarditis	3/4 (1 het, 2 lym, 1 pvc)	3/5 (1 pvc/swe, 2 lym/int/±pvc)	2/3 (1 lym, 1 nec)	0/3	0/1
Pneumonia	3/4 (3 mix)	3/5 (2 lym/int, 1 mix)	2/3 (2 lym/int)	3/3 (2 het, 1 mix)	1/1 (1 lym/int/nec)
Kidney epithelial necrosis	1/3	4/5	1/3	0/3	0/1
Hepatitis	4/4 (4 mix)	4/5 (4 mix±nec)	3/3 (1 lym/nec, 2 mix)	2/2 (1 mix/nec, 1 het)	1/1 (1 nec)
Splenitis	2/3 (2 mix)	4/5 (3 mix, 1 nec)	1/2 (1 lym/nec)	3/3 (1 mix/nec, 2 nec)	1/1 (1 nec)
Haemosiderosis	2/4	4/5	1/3	2/3	0/1
Skin cloaca dermatitis	2/4 (2 mix)	4/4 (2 mix, 2 lym)	1/2 (1 lym)	0/3	0/1

Deg: degeneration of white matter; gli: satellitosis, gliosis; het: heterophilic infiltrates; int: interstitial infection; lym: lymphoplasmacytic infiltrates (lymphocytes, plasma cells, histiocytes); mix: mixed infiltrates; nec: necrosis; pvc: perivascular cuffing; rfsh: retained feather shafts; Sn: *Strix nebulosa*; swe: endothelial cell swelling; Tm: *Turdus merula*; USUV: Usutu virus.

[a] *Plasmodium* infection was determined by cytology and histology, USUV infection by RT-PCR test on brain, spleen, heart and/or liver.

[b] Number of cases positive/total number of cases examined.

[c] Incidental findings included gastrointestinal worms in eight of 12 blackbirds and a mycotic infection in the glandular stomach of one owl.

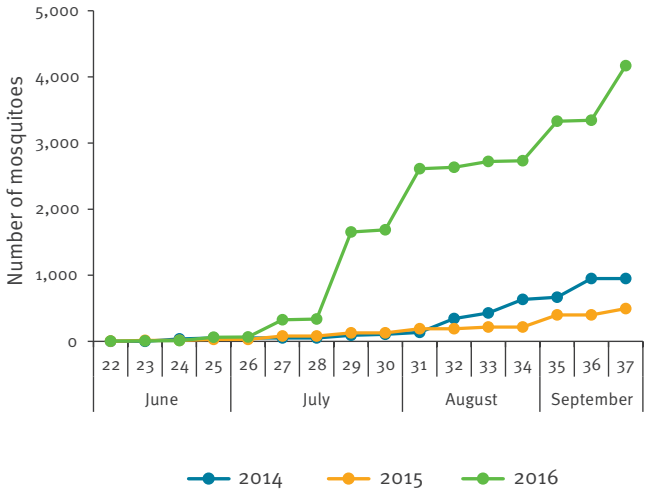
with lymphoplasmacytic inflammation [9,14-17]. In this outbreak, the pathological findings raised two questions. Firstly, many of the birds were co-infected with *Plasmodium* spp. Mosquitoes are the vectors of both USUV and *Plasmodium* spp., which may explain the high number of dual infections. Alternatively, a fatal outcome of USUV infection may be more probable in co-infection. Secondly, while skin lesions during USUV outbreaks have been reported earlier [9,18], causal association is unknown and needs to be studied.

We used citizen science data to identify the area where the virus probably circulated most intensively up to 23 September 2016. Infected blackbirds maintain virus circulation [15], and the observed pattern will partly reflect the density of resident blackbird populations. Ongoing wild bird counts will provide insight into the impact of USUV on resident bird populations.

The emergence of USUV in the Netherlands illustrates the continuous geographical expansion of zoonotic arboviruses in Europe, documented elsewhere [8]. It serves as another warning of the expanding geographical range of regions suitable for sustained arbovirus circulation. In areas with endemic circulation, human infections seem to occur very rarely with only 13 human cases described in literature until now [19]. Human clinical cases present with neurological signs, fever, rash, jaundice or combinations thereof. Subclinical human USUV infections are a concern in blood transfusions or organ transplants [20], and recent data from Italy suggest that subclinical cases in regions with sustained

Figure 2

Cumulative number of mosquitoes found per year, at the sites of four used tyre companies, the Netherlands, week 22 to week 37 (end of May to mid-September)



The tyre companies were located throughout the country: Leeuwarden (north), Amsterdam (west), Tilburg (south) and Nederweert (east).

USUV circulation may be more common than previously thought [19]. The same study showed that USUV was the cause of previously unexplained encephalitis cases [19], indicating that USUV should be included in the differential diagnosis of such cases in endemic areas. These recent public health findings suggest that USUV diagnostic capability and adequate USUV surveillance with molecular typing are warranted in regions shown to be suitable for USUV circulation. Although the 2016 mosquito season is coming to an end, physicians should be aware of putative USUV infection in cases of viral encephalitis of unknown aetiology, and vigilance should be maintained in the coming mosquito season in 2017.

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Conflict of interest

None declared.

Authors' contributions

Manuscript writing JMR, MLK, RPBF, AS and CBEMR. Pathology MLK, JI and AG. Virology CBEMR. Live bird surveillance CBEMR, HVDJ and MGPK. Common blackbird density data RPBF. Mosquito abundance data AS. Citizen science data JMR, RS, RPBF and JS. All authors critically read the manuscript.

References

1. Reusken C, Zutt I, Kik M, Cleton N, Rijks J, Schmidt-Chanasit J, et al. [No proof for usutuvirus as cause of death in songbirds in the Netherlands (fall 2012)]. Tijdschr Diergeneeskd. 2014;139(3):28-30. Dutch. PMID:24701786PMID: 24701786
2. 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. DOI: 10.1128/JCM.39.5.1922-1927.2001 PMID: 11326014
3. Vanstreels RE, Capellino F, Silveira P, Braga ÉM, Rodríguez-Heredia SA, Loureiro J, et al. Avian Malaria (Plasmodium spp.) in Captive Magellanic Penguins (Spheniscus magellanicus) from Northern Argentina, 2010. J Wildl Dis. 2016;52(3):734-7. DOI: 10.7589/2015-08-219 PMID: 27285418
4. Dinhopl N, Nedorost N, Mostegl MM, Weissenbacher-Lang C, Weissenböck H. In situ hybridization and sequence analysis reveal an association of Plasmodium spp. with mortalities in wild passerine birds in Austria.Parasitol Res. 2015;114(4):1455-62. DOI: 10.1007/s00436-015-4328-z PMID: 25636246
5. Schekkerman H, van Turnhout C, van Kleunen A, van Diek H, Altenburg J. Towards a new Dutch bird atlas: design of the fieldwork in 2012-2015.Limosa.2012;85:133-41.
6. Weissenböck H, Bakonyi T, Rossi G, Mani P, Nowotny N. Usutu virus, Italy, 1996.Emerg Infect Dis. 2013;19(2):274-7. DOI: 10.3201/eid1902.121191 PMID: 23347844
7. Engel D, Jöst H, Wink M, Börstler J, Bosch S, Garigliany MM, et al. Reconstruction of the Evolutionary History and Dispersal of Usutu Virus, a Neglected Emerging Arbovirus in Europe and Africa. MBio. 2016;7(1):e01938-15. DOI: 10.1128/mBio.01938-15 PMID: 26838717
8. Nikolay B. A review of West Nile and Usutu virus co-circulation in Europe: how much do transmission cycles overlap?Trans R Soc Trop Med Hyg. 2015;109(10):609-18. DOI: 10.1093/trstmh/trv066 PMID: 26286946
9. Becker N, Jöst H, Ziegler U, Eiden M, Höper D, Emmerich P, et al. Epizootic emergence of Usutu virus in wild and captive birds in Germany. PLoS One. 2012;7(2):e32604. DOI: 10.1371/journal.pone.0032604 PMID: 22389712
10. Calzolari M, Gaibani P, Bellini R, Defilippo F, Pierro A, Albieri A, et al. Mosquito, bird and human surveillance of West Nile and Usutu viruses in Emilia-Romagna Region (Italy) in 2010. PLoS One. 2012;7(5):e38058. DOI: 10.1371/journal.pone.0038058 PMID: 22666446
11. Ibañez-Justicia A, Stroo A, Dik M, Beeuwkes J, Scholte EJ. National Mosquito (Diptera: Culicidae) Survey in The Netherlands 2010-2013.J Med Entomol. 2015;52(2):185-98. DOI: 10.1093/jme/tju058 PMID: 26336303
12. Royal Netherlands Meteorological Institute (KNMI). Recordnatte juni in het zuidoosten. [Record wet June month in the south-east]. De Bilt: KNMI; 14 July 2016. Dutch. Available from: https://www.knmi.nl/over-het-knmi/nieuws/recordnatte-juni-in-het-zuidoosten
13. Royal Netherlands Meteorological Institute (KNMI). September 2016. Zeer warm, zeer droog en zeer zonnig. [September 2016. Very warm, dry and sunny]. De Bilt: KNMI; September 2016. Dutch. Available from: https://www.knmi.nl/nederland-nu/klimatologie/maand-en-seizoensoverzichten/2016/september

14. Weissenböck H, Kolodziejek J, Url A, Lussy H, Rebel-Bauder B, Nowotny N. Emergence of Usutu virus, an African mosquito-borne flavivirus of the Japanese encephalitis virus group, central Europe.Emerg Infect Dis. 2002;8(7):652-6. DOI: 10.3201/eid0807.020094 PMID: 12095429
15. Chvala S, Kolodziejek J, Nowotny N, Weissenböck H. Pathology and viral distribution in fatal Usutu virus infections of birds from the 2001 and 2002 outbreaks in Austria.J Comp Pathol. 2004;131(2-3):176-85. DOI: 10.1016/j.jcpa.2004.03.004 PMID: 15276857
16. Steinmetz HW, Bakonyi T, Weissenböck H, Hatt JM, Eulenberger U, Robert N, et al. Emergence and establishment of Usutu virus infection in wild and captive avian species in and around Zurich, Switzerland--genomic and pathologic comparison to other central European outbreaks. Vet Microbiol. 2011;148(2-4):207-12. DOI: 10.1016/j.vetmic.2010.09.018 PMID: 20980109
17. Höfle U, Gamino V, de Mera IG, Mangold AJ, Ortíz JA, de la Fuente J. Usutu virus in migratory song thrushes, Spain.Emerg Infect Dis. 2013;19(7):1173-5. DOI: 10.3201/eid1907.130199 PMID: 23764143
18. Bosch S. Zerzaust, gerupft oder glatzköpfig: Gefiederauffälligkeiten bei Amseln Turdus merula infolge einer Infektion mit Usutu-Viren? [Tousled, plucked or bald: feather anomalies in blackbirds Turdus merula following infection with Usutu viruses?]. Ornithologische Mitteilungen.2012;64(11/12):305-16. German.
19. Grottola A, Marcacci M, Tagliazucchi S, Gennari W, Di Gennaro A, Orsini M, et al. Usutu virus infections in humans: a retrospective analysis in the municipality of Modena, Italy. Clin Microbiol Infect. 2016;pii: S1198-743X(16)30425-6. doi: DOI: 10.1016/j.cmi.2016.09.019 .
20. Allering L, Jöst H, Emmerich P, Günther S, Lattwein E, Schmidt M, et al. Detection of Usutu virus infection in a healthy blood donor from south-west Germany, 2012. Euro Surveill. 2012;17(50):20341.PMID: 23241231

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Outbreak of *Neisseria meningitidis* capsular group W among scouts returning from the World Scout Jamboree, Japan, 2015

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The 23rd World Scout Jamboree was held in Japan from 28 July to 8 August 2015 and was attended by over 33,000 scouts from 162 countries. An outbreak of invasive meningococcal disease capsular group W was investigated among participants, with four confirmed cases identified in Scotland, who were all associated with one particular scout unit, and two confirmed cases in Sweden; molecular testing showed the same strain to be responsible for illness in both countries. The report describes the public health action taken to prevent further cases and the different decisions reached with respect to how wide to extend the offer of chemoprophylaxis in the two countries; in Scotland, chemoprophylaxis was offered to the unit of 40 participants to which the four cases belonged and to other close contacts of cases, while in Sweden chemoprophylaxis was offered to all those returning from the Jamboree. The report also describes the international collaboration and communication required to investigate and manage such multinational outbreaks in a timely manner.

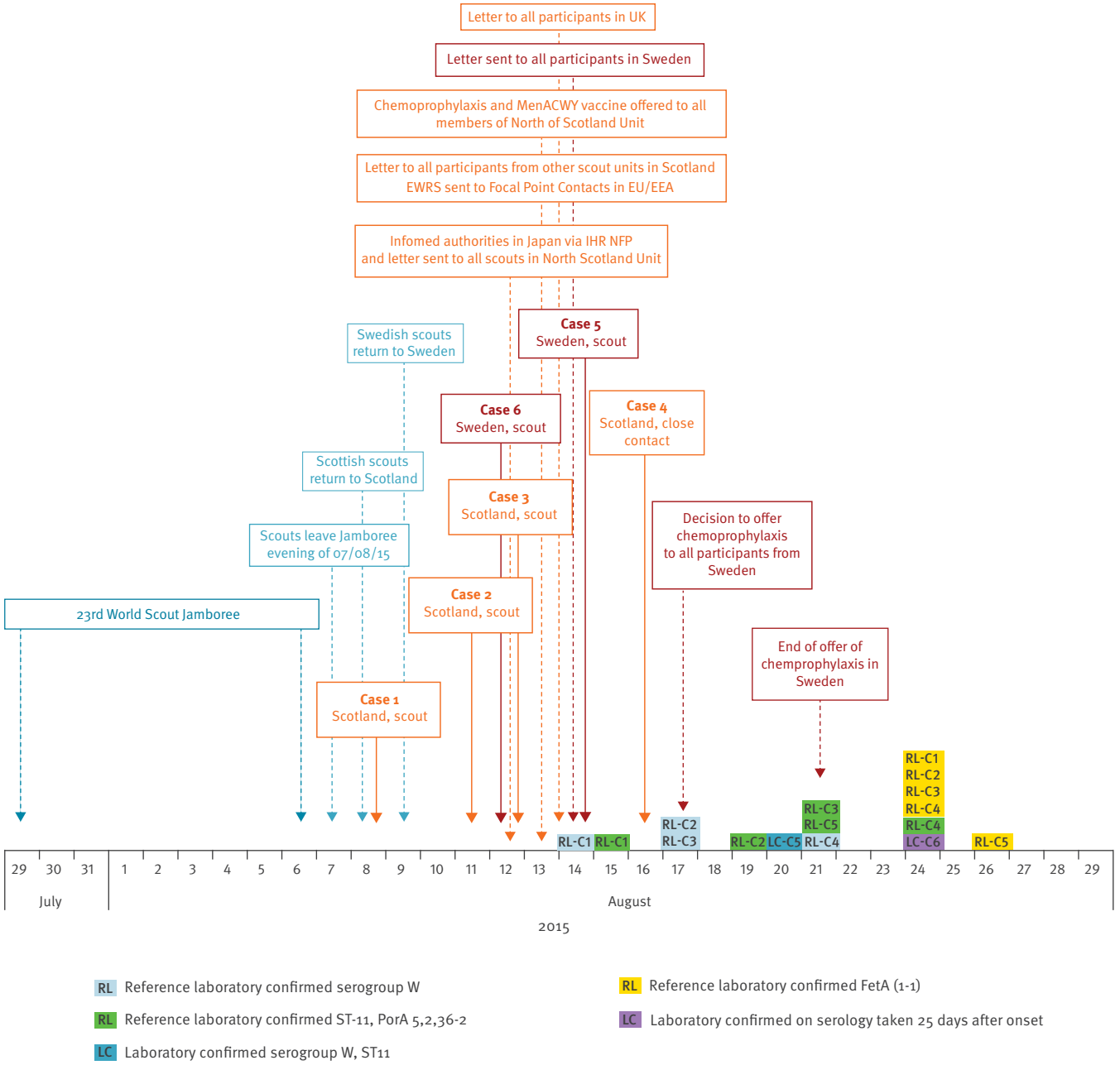
Introduction

Definitions of mass gatherings vary greatly, with some sources categorising any gathering of more than 1,000 individuals as a mass gathering, while others require the attendance of as many as 25,000 people to qualify. Irrespective of the definition, mass gatherings represent large numbers of people attending an event that is focused at specific sites for a finite time [1]. Mass gatherings provide the potential for disseminated outbreaks for a range of pathogens, especially respiratory and gastrointestinal [2-4]. Although meningococcal outbreaks are rarely reported from mass gatherings, there have been previous examples. An outbreak of meningococcal capsular group C was observed with 11 linked cases following a youth football tournament held in Belgium in 1997 [5]. The Hajj pilgrimages in 2000 and 2001 were associated with outbreaks of meningococcal capsular group W (MenW) [6], with a high attack rate among pilgrims and their household contacts [7]. Among those affected by the outbreak strain in England and Wales, the case fatality ratio (CFR) was 20%, significantly higher than the CFR of 9% for all other culture-confirmed cases of meningococcal disease reported in England and Wales between 1995 and 2000 [8]. In response to the outbreak in 2000, the United Kingdom (UK) Department of Health recommended MenACWY vaccine for those attending the Hajj [8].

The 23rd World Scout Jamboree was held in Yamaguchi City, Yamaguchi Prefecture, Japan from 28 July to 8 August 2015 and was attended by over 33,000 scouts from 162 countries. This included 160 scouts and 108 adults from Scotland who were either leaders or part of the international support staff. The scouts attending from Scotland comprised five distinct units, one of which was the North of Scotland unit, with 36 scouts and four adult leaders, 60% of whom were male (16 females and 24 males). The mean age of the scouts in this unit was 16.4 years (range 15–17 years).
On 12 August 2015, Health Protection Scotland was informed by a Health Protection Team in the North of

Figure 1

Timeline for confirmed cases of meningococcal infection among scouts returning from the World Scout Jamboree, Scotland and Sweden, 28 July to 29 August 2015 (n=6)



CSF: cerebrospinal fluid; EEA: European Economic Area; EU: European Union; EWRS: European Early Warning and Response System; IHR NFP: International Health Regulations National Focal Point; ref lab: reference laboratory; UK: United Kingdom.

Scotland of a laboratory-confirmed case of invasive meningococcal disease in a scout belonging to the North of Scotland unit who had attended the 23rd World Scout Jamboree.

Epidemiological investigation

The epidemiological investigation carried out is illustrated in the Figure.

CSF: cerebrospinal fluid; EEA: European Economic Area; EU: European Union; EWRS: European Early Warning and Response System; IHR NFP: International Health Regulations National Focal Point; ref lab: reference laboratory; UK: United Kingdom.

Public health action in the United Kingdom

Following the identification of the first laboratory-confirmed case (12 August), an alert was sent via email to all Health Protection Teams in Scotland to raise awareness, an information letter emailed to all scouts and leaders in the North of Scotland unit and information passed to the authorities in Japan via the UK

International Health Regulations National Focal Point (IHR NFP), Public Health England. The public health response was managed according to the UK meningococcal guidance [9].

The first Incident Management Team (IMT) met on 13 August with representatives from the North of Scotland National Health Service (NHS) Boards Health Protection Teams, microbiology, infectious diseases, Scottish Government and Health Protection Scotland. Five subsequent IMTs were held with additional representation from Public Health England and Public Health Agency Northern Ireland.

Following the identification on 13 August of the second laboratory-confirmed case and the identification of four other possible cases among scouts from the North of Scotland unit a risk assessment was undertaken. The North of Scotland unit appeared to be a self-contained unit within the UK contingent travelling to and from the Jamboree. Furthermore, their tent accommodation at the site was not adjacent to the other UK scout units. Chemoprophylaxis with ciprofloxacin and MenACWY conjugate vaccine was then offered to all scouts and leaders in the North of Scotland unit in addition to other close contacts of the two confirmed cases. The decision to recommend MenACWY vaccine was based on the results of preliminary antigen detection tests from the local hospital laboratory for case one. All individuals received chemoprophylaxis, and were offered vaccination, by the end of 14 August and 18 August, respectively. Interviews conducted with these two cases did not initially identify any close contacts outside the North of Scotland unit (even for their return international flight) and the immediate households of the confirmed cases. However, a close contact of the first case in a scout unit in another area of the UK was subsequently identified on the evening of 13 August, and received chemoprophylaxis that night and MenACWY vaccine on 14 August. On the evening of 13 August an information letter was emailed to all scouts from Scotland who attended the Jamboree, providing information about the incident.

On 14 August, one of the four possible cases under investigation was confirmed to have invasive meningococcal disease and a further two possible cases identified. This brought the total to three confirmed and five possible cases. In the absence of additional information indicating joint activities with other scout groups from the Jamboree, the IMT reiterated their decision not to offer antibiotic prophylaxis to any of the other UK scouts. Following the identification of the third confirmed case, there was discussion as to whether MenACWY vaccine should be offered to all participants in the UK. A decision was made not to extend the offer of vaccination, based on no evidence of spread in the UK beyond the North of Scotland unit. Additional considerations supporting this decision included the practicalities of providing timely immunisation to such a large cohort, and concerns about vaccine availability, since a MenACWY immunisation programme had just commenced phased introduction for all 14–18 year olds and new university entrants in the UK [10]. However, using contact details provided by Scouts UK, an information letter outlining the situation and the action to take in the event of symptom development was emailed that day to the parents/guardians of all scouts, leaders and international support staff who attended the Jamboree from across the UK (ca 4,000). The rationale for this letter was that all such individuals were within the incubation period for meningococcal disease, being within 7 days of return to the UK from the Jamboree. For those individuals with no email address or for whom an undeliverable or out-of-office email response was received, alternative contact details (phone and/or postal address) were provided to the Health Protection agencies of each UK country to allow further attempts to provide information about the incident.

On 17 August, a fourth case was confirmed in a close contact of one of the scouts from the North of Scotland Unit. That scout was not a case and had reported no close contact with any of the other three confirmed cases. Chemoprophylaxis and MenACWY vaccine was provided to the close contacts of the scout's infected close contact, including re-issuing chemoprophylaxis to the scout, due to continuing contact with the infected close contact following initial chemoprophylaxis.

Confirmed cases had a range of presenting symptoms (Figure). It was observed that a number of cases had respiratory symptoms such as cough or sore throat. As a result it was decided that there should be a low threshold for treating possible cases who presented with respiratory symptoms.

None of the remaining five possible cases under investigation were confirmed as invasive meningococcal disease or, on further review, clinically considered to be a case of invasive meningococcal disease; one had a positive throat swab for group G streptococci, one a positive throat swab for rhinovirus/enterovirus (combined test does not determine which is positive). All four confirmed cases made a rapid clinical recovery after admission to hospital and were discharged from hospital by 20 August.

A total of 53 individuals in Scotland and one outside Scotland received chemoprophylaxis and were offered vaccination.

Microbiological investigations in Scotland

Neisseria meningitidis isolates submitted to the Scottish *Haemophilus*, *Legionella*, Meningococcus and Pneumococcus Reference Laboratory (SHLMPRL) by regional diagnostic microbiology laboratories were characterised by standard phenotypic procedures (api NH (BioMérieux), Wellcogen *N. meningitidis* ACYW135 latex reagent (Remel Europe Ltd.) and monovalent meningococcus agglutinating serum (Remel Europe Ltd.)). Confirmation of *N. meningitidis* isolates and

detection of *N. meningitidis* DNA in clinical specimens was determined by *ctrA* PCR [11]. Genotypic capsular grouping was performed by *siaD* PCR [12] *siaDW135* and *siaDY* primer and probe information was kindly provided by Dr Malcolm Guiver at the PHE Meningococcal Reference Unit (Manchester, UK). Multilocus sequence typing (MLST), PorA variable region (VR) sequencing and FetA VR sequencing were performed as outlined on the *Neisseria* sequence-typing website [13].

All four isolates from the confirmed cases were indistinguishable by the phenotypic and molecular typing procedures outlined above. Based upon the EMGM-recommended strain designation [14] this identified the *N. meningitidis* strain as W: P1.5,2,36-2: F1-1: ST-11 (cc11).

Preliminary typing suggested that the W strain is indistinguishable from that responsible for the recent increase in MenW ST-11 disease in England and Wales since 2009 and more recent indications of increased disease in Scotland, with 15 isolates of MenW ST-11 reported in Scotland in the first 45 weeks of 2015, accounting for 24% of all isolates over the time period, compared with just five, accounting for 7% of cases in 2014 (SHLMPRL, data not shown)

International aspects

On the evening of 13 August, a European Early Warning and Response System (EWRS) message was circulated to the National Focal Point contacts in the European Union (EU)/European Economic Area (EEA) about the meningococcal cases in Scotland.

On 14 August the Public Health Agency of Sweden, with the help of the Swedish Scout Organisation, distributed a letter to the returning scouts recommending them to seek healthcare promptly upon signs of meningitis illness. On Sunday 16 August, the first Swedish case (case 5, Figure), a scout who had attended the Jamboree, was admitted to hospital with a clinical picture of meningitis and shock, with date of onset of first symptoms (not including shock) of 14 August. The case was treated in intensive care for 6 days. The case finally recovered well and was discharged on 28 August. Gram-negative diplococci were initially found in CSF and *N. meningitidis* was verified by PCR the next day and subsequently by culture. The isolated strain was confirmed as capsular group W with the PorA profile 5,2,36-2, the same as identified from the four Scottish cases. This strain had also been previously isolated in Sweden in 2014 and 2015.

On the evening of 16 August the Public Health authorities received reports of a second suspected case, a scout leader who became unwell on 13 August and was hospitalised on 15 August due to suspected septicaemia. The cases and their close contacts were managed according to the national guidance [15,16].

On the morning of 17 August, an urgent teleconference was convened by members of Communicable Disease Control and Prevention in Stockholm and Gothenburg, the Public Health Agency of Sweden and the National reference laboratory for Pathogenic *Neisseria* in Örebro, Sweden. During the one-hour meeting, two more suspected cases of meningococcal septicaemia were reported; one scout hospitalised in the South of Sweden and one scout hospitalised in Stockholm. This latter case was positive for *N. meningitidis* capsular group W with PorA profile 5,2,36-2 in throat swab (result available on 26 August) and later confirmed by serology (case 6, Figure). Thus at that point in time the authorities in Sweden were aware of one confirmed and three suspected cases that appeared to be from different units and all hospitalised within 24 hours. The authorities were therefore unable to define a limited high-risk group among the scouts. Further, the cases had occurred within a high-risk setting for transmission with young people living in camp conditions and close social interaction. Hence a decision was taken to recommend ciprofloxacin prophylaxis to all 1,900 scouts across Sweden. It was also decided when possible to obtain a throat swab to find out the carriage rate in such an outbreak, as this had not had been investigated in Sweden in modern times and there was now a unique opportunity to find out the carrier state of meningococci in teenagers in Sweden, a low-incidence country for invasive meningococcal disease (0.5/100,000 inhabitants in 2014). These screening results will be published at a later date.

The offer of free prophylactic antibiotics in Sweden ended on 21 August, after which the risk of further cases due to transmission in Japan was deemed to be very unlikely. On the same day a questionnaire was administered to all participants in order to determine how many had taken up the offer, and how many had a throat swab taken, in addition to assessing general satisfaction with information and service delivery. Data from Sweden indicate that chemoprophylaxis uptake was around 80%, and that more than 90% were satisfied with the information and instructions provided by the authorities. However, there were reports from healthcare providers in Sweden that the information about the intervention had not been received in all clinics.

Follow-up of the two confirmed Swedish cases identified that they belonged to the same scout unit. This information had not been available when the decision to offer chemoprophylaxis was made.

The first confirmed Swedish case was later identified as having attended a cultural day at the campsite on 2 August. The cultural exchange day comprised an inter-faith ceremony and a food festival in the afternoon during which scouts cooked their own traditional dishes and invited scouts from other countries to taste and experience food and cultural differences among countries. Scouts from all countries were asked to walk

around the sub-camp to mingle and taste food from different countries, and during this event they visited the North of Scotland unit and tried their food and drink.

The organisers also held discotheques every third evening during the Jamboree. Anecdotal evidence also suggested extensive mixing between participants from many countries in keeping with the international nature of the meeting.

Examination of the campsite plan revealed that the North of Scotland unit had slept in tents in the western hub of the camp, as had the two confirmed cases from Sweden, although they were not immediately adjacent. The units closest to the North of Scotland unit were from the United States, Hong Kong, Japan, France, Luxembourg and Pakistan, none of which reported any cases

The two possible cases from Sweden were negative for *N. meningitidis* and subsequently discounted by the authorities as meningococcal disease.

Throughout the investigation, regular updates were issued via EWRS to EU/EEA countries, and information exchanged, through the IHR NFP, with authorities in Japan. The EWRS alerts provided a rapid mechanism for both disseminating and collating information. In response to the EWRS, 20 countries reported that they had issued information to participants to raise awareness of the signs and symptoms. None of these 20 countries recommended antibiotics and no associated meningococcal cases were reported.

The Ministry of Health, Labour and Welfare in Japan requested that the Scout Association of Japan alert participants to be aware of the signs and symptoms of meningococcal disease and liaised with the Jamboree organisers. The Jamboree organiser provided information to units who had stayed near the North of Scotland unit. There were no cases of invasive meningococcal disease reported in Japan associated with the Jamboree.

Discussion

The North of Scotland scouts were not vaccinated against MenW before the Jamboree, since the UK recommendations on immunisation of travellers do not include Men ACWY vaccination in these circumstances. Likewise, neither of the Swedish cases was vaccinated. However, although vaccination is not recommended in Sweden before mass gathering events, a small number of Swedish scouts had been vaccinated before the trip.

In response to a recent UK increase in MenW disease, in February 2015 the UK advisory body on immunisation the Joint Committee on Vaccination and Immunisation recommended a vaccination programme aimed at protecting adolescents against meningococcal capsular groups ACW and Y strains. This was felt to be the best option to generate population-level protection since teenagers are in the age group with highest meningococcal carriage levels, and also an age group at increased risk of disease [17]. This recommendation was accepted by the UK Departments of Health. The immunisation programme in Scotland for 14–18-year-olds started in August 2015 for young people who had left school, whether attending full-time education or not, and others aged < 25 years starting university for the first time, and the school-based programme began in January 2016 [10] This programme has replaced the earlier MenC immunisation offered as an adolescent booster in schools with a catch-up programme. Therefore, in future years, adolescents from the UK attending Jamborees and similar mass-gathering events should be protected against these capsular groups. As similar programmes are not currently in place in all other countries it will be for individual countries to consider whether there should be local recommendations for those attending such events.

The rapid communication of the identification of meningococcal disease among participants of a dispersed mass gathering allowed public health authorities to target information to the international Scout Movement attendees of the Jamboree in individual countries. This timely dissemination led to rapid identification of other Scottish cases, and facilitated the identification of an epidemiological link to the Swedish case. In both the UK and Sweden the excellent electronic records and cooperation of scouting organisations greatly facilitated this process and allowed the rapid dissemination of information to participants. However it is recognised that for many mass gatherings where similar outbreaks may occur, for example music festivals, sports events and religious celebrations, such comprehensive contact lists will not be available, making it extremely difficult to identify and contact potentially exposed individuals within the critical incubation window.

It was of interest that the IMT in Scotland and the rest of the UK arrived at different decisions than Sweden in terms of the extent of chemoprophylaxis offered. In Scotland the risk assessment for the cases, which were restricted to the North of Scotland, limited this offer to a small group, whereas in Sweden all 1,900 Jamboree attendees were offered chemoprophylaxis as it was not possible to identify a specific cohort at increased risk, as the information available on 17 August suggested four cases under investigation hospitalised in the previous 24 hours from different units.

Uptake of chemoprophylaxis was high in both Scotland and Sweden and administered in a timely manner. Unfortunately it is not possible to determine if the mass distribution of prophylaxis prevented further cases. Comments from Sweden that not all healthcare providers had received the appropriate information highlight the importance of clear communication channels between public health institutions and healthcare systems, and the practical issues of conducting large exercises with tight timescales

In a previous analysis of 129 UK MenW cases, none were contacts of another MenW case [18], making this the largest cluster (n=6 confirmed cases; 4 in Scotland) in this current UK increase in MenW disease. Most individuals infected with *N. meningitidis* experience a period of asymptomatic carriage with no disease. A meta-analysis of carriage prevalence has shown increased carriage throughout childhood from 4.5% in infants to a peak of 23.7% in 19 year-olds subsequently decreasing in adulthood to 7.8% in 50 year-olds [19], similar levels for 19–25 year-olds of 26.5% were reported from a UK carriage study in 2011, with capsular groups B and Y the most common at that time [20]. Although meningococcal carriage is potentially high in the participant age group, with carriage also depending on exposure to smoking, intimate kissing, pub-/club-type social settings and coincident respiratory tract infections of viral or bacterial origin [21], it is unclear why these cases developed invasive disease. Extensive social mixing associated with the Jamboree, preceding viral/bacterial infection and long-haul air travel could have been contributing factors.

Evidence from the UK increase of this sequence type (ST11), has suggested an often atypical clinical presentation, with initially mild symptoms for some cases, and a case fatality rate of 12% [18], lower than that previously reported from the MenW outbreak associated with the Hajj [8]. The four confirmed cases from Scotland tended to have an atypical presentation, dominated by respiratory symptoms and did not have a severe course of disease, with none requiring intensive care admission. It is possible the latter may reflect early clinical presentation in response to the public health alert and prompt antibiotic intervention. Interestingly, in the previous analysis of UK MenW cases, such respiratory presentations were also associated with less severe disease [18]. Whole-genome sequencing is underway to further characterise the outbreak isolates from Scotland and allow more detailed comparison with the endemic UK strain. These data may help explain apparent associations between clinical presentation, severity and outcome. Continued enhanced surveillance in this area will be important.

Public health recommendation: decisions on the need for mass prophylaxis and vaccination

Decisions on the need for mass prophylaxis in large events like this need to be taken rapidly, even if only limited information is available initially. Each situation is likely to be different and will not be predictable. While UK guidance addresses such contingency, further work on development of generic decision algorithms should be considered. If a decision is taken to recommend prophylaxis or vaccination it is important that information can be delivered quickly, within hours to both those who may be at risk, and also to the healthcare system so they can arrange for delivery of the service in a timely manner.

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Conflict of interest

None declared.

Authors' contributions

Representatives from all agencies represented on the incident management team and investigation (ASP, KO, DW, ST, KS, GS, BP, AL, AW, HF, MW and JM) prepared the manuscript on behalf of the Incident Management Team. All authors have seen and approved the final manuscript.

References

1. Memish ZA, Stephens GM, Steffen R, Ahmed QA. Emergence of medicine for mass gatherings: lessons from the Hajj. Lancet Infect Dis. 2012;12(1):56-65. DOI: 10.1016/S1473-3099(11)70337-1 PMID: 22192130
2. Abubakar I, Gautret P, Brunette GW, Blumberg L, Johnson D, Poumerol G, et al. Global perspectives for prevention of infectious diseases associated with mass gatherings. Lancet Infect Dis. 2012;12(1):66-74. DOI: 10.1016/S1473-3099(11)70246-8 PMID: 22192131
3. Duizer E, Timen A, Morroy G, de Roda Husman AM. Norovirus outbreak at an international scout Jamboree in the Netherlands, July-August 2004: international alert. Euro Surveill. 2004;8(33):2523.
4. Tabatabaei SM, Metanat M. Mass gatherings and infectious diseases epidemiology and surveillance. International Journal of Infection 2015;2(2): e22833. Available from: http://intjinfection.com/?page=article&article_id=22833
5. Reintjes R, Kistemann T, MacLehose L, McKee M, Gill N, Weinberg J, et al. Detection and response to a meningococcal disease outbreak following a youth football tournament with teams from four European countries. Int J Hyg Environ Health. 2002;205(4):291-6. DOI: 10.1078/1438-4639-00156 PMID: 12068748
6. Lingappa JR, Al-Rabeah AM, Hajjeh R, Mustafa T, Fatani A, Al-Bassam T, et al. Serogroup W-135 meningococcal disease during the Hajj, 2000. Emerg Infect Dis. 2003;9(6):665-71. DOI: 10.3201/eid0906.020565 PMID: 12781005
7. Wilder-Smith A, Barkham TM, Ravindran S, Earnest A, Paton NI. Persistence of W135 Neisseria meningitidis carriage in returning Hajj pilgrims: risk for early and late transmission to household contacts.Emerg Infect Dis. 2003;9(1):123-6. DOI: \\ PMID: 12533295
8. Hahné SJ, Gray SJ, Aguilera J-F, Crowcroft NS, Nichols T, et al. W135 meningococcal disease in England and Wales associated with Hajj 2000 and 2001. Lancet. 2002;359(9306):582-3. DOI: 10.1016/S0140-6736(02)07716-4 PMID: 11867116
9. Health Protection Agency (HPA). Guidance for public health management of meningococcal disease in the UK. London: HPA; 2012. [Accessed 19 Nov 2015]. Available from: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/322008/Guidance_for_management_of_meningococcal_disease_pdf.pdf
10. Chief Medical Officer Directorate letter. Meningococcal ACWY (Men ACWY) vaccination programme: University Freshers and adolescents aged 14-18. Edinburgh: Scottish Government Health Directorate 20 August 2015. Available from: www.sehd.scot.nhs.uk/cmo/CMO(2015)15.pdf
11. Guiver M, Corless CE, Marsh WJ, Gray SJ, Newbold LS, Borrow R, et al. Modifications to a published ctrA PCR assay for the improved non-culture confirmation of meningococcal disease in England and Wales. Poster P082. 11th congress EGM 2011. European Meningococcal Disease Society 2011. Available from: www.meningitis.org/assets/x/53939
12. Pollard AJ, Maiden MCJ, editors. Meningococcal disease: methods and protocols. Totowa, NJ: Humana Press; 2001.
13. TypingNMLS. Oxford: University of Oxford. [Accessed 1 Aug 2015]. Available from: http://pubmlst.org/neisseria
14. Jolley KA, Brehony C, Maiden MC. Molecular typing of meningococci: recommendations for target choice and nomenclature.FEMS Microbiol Rev. 2007;31(1):89-96. DOI: 10.1111/j.1574-6976.2006.00057.x PMID: 17168996
15. Smittskyddsläkarföreningen. Meningokocksjukdom (invasiv) läkarinformation. [Invasive meningococcal disease: medical information] 11 Oct 2015. Swedish. Available from: https://www.slf.se/Foreningarnas-startsidor/Intresseforening/Smittskyddslakarforeningen/Smittskyddsblad-/Meningokocksjukdom-invasiv-lakarinformation-2014-11-10/
16. Folkhälsomyndigheten (The Public Health Agency of Sweden). Rekommendationer för profylax kring fall av invasive meningokockinfektion. [Recommendations for prophylaxis regarding cases of invasive meningococcal disease]. Solna: Folkhälsomyndigheten. December 2008. Swedish. Available from: https://www.folkhalsomyndigheten.se/publicerat-material/publikationsarkiv/r/Rekommendationer-for-profylax-kring-fall-av-invasiv-meningokockinfektion/
17. Joint Committee on Vaccination and Immunisation (JCVI). Minutes of the meeting on 4 February London: JCVI; 2015. Available from: https://www.gov.uk/government/groups/joint-committee-on-vaccination-and-immunisation
18. Ladhani SN, Beebeejaun K, Lucidarme J, Campbell H, Gray S, Kaczmarski E, et al. Increase in endemic Neisseria meningitidis capsular group W sequence type 11 complex associated with severe invasive disease in England and Wales. Clin Infect Dis. 2015;60(4):578-85. DOI: 10.1093/cid/ciu881 PMID: 25389259
19. Christensen H, May M, Bowen L, Hickman M, Trotter CL. Meningococcal carriage by age: a systematic review and meta-analysis.Lancet Infect Dis. 2010;10(12):853-61. DOI: 10.1016/S1473-3099(10)70251-6 PMID: 21075057
20. Jeppesen CA, Snape MD, Robinson H, Gossger N, John TM, Voysey M, et al. Meningococcal carriage in adolescents in the United Kingdom to inform timing of an adolescent vaccination strategy. J Infect. 2015;71(1):43-52. DOI: 10.1016/j.jinf.2015.02.006 PMID: 25709085
21. MacLennan J, Kafatos G, Neal K, Andrews N, Cameron JC, Roberts R, et al. , United Kingdom Meningococcal Carriage Group. Social behavior and meningococcal carriage in British teenagers.Emerg Infect Dis. 2006;12(6):950-7. DOI: 10.3201/eid1206.051297 PMID: 16707051

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Research article

An international invasive meningococcal disease outbreak due to a novel and rapidly expanding serogroup W strain, Scotland and Sweden, July to August 2015

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The 23rd World Scout Jamboree in 2015 took place in Japan and included over 33,000 scouts from 162 countries. Within nine days of the meeting ending, six cases of laboratory-confirmed invasive serogroup W meningococcal disease occurred among scouts and their close contacts in Scotland and Sweden. The isolates responsible were identical to one-another by routine typing and, where known (4 isolates), belonged to the ST-11 clonal complex (cc11) which is associated with large outbreaks and high case fatality rates. Recent studies have demonstrated the need for high-resolution genomic typing schemes to assign serogroup W cc11 isolates to several distinct strains circulating globally over the past two decades. Here we used such schemes to confirm that the Jamboree-associated cases constituted a genuine outbreak and that this was due to a novel and rapidly expanding strain descended from the strain that has recently expanded in South America and the United Kingdom. We also identify the genetic differences that define the novel strain including four point mutations and three putative recombination events involving the horizontal exchange of 17, six and two genes, respectively. Noteworthy outcomes of these changes were antigenic shifts and the disruption of a transcriptional regulator.

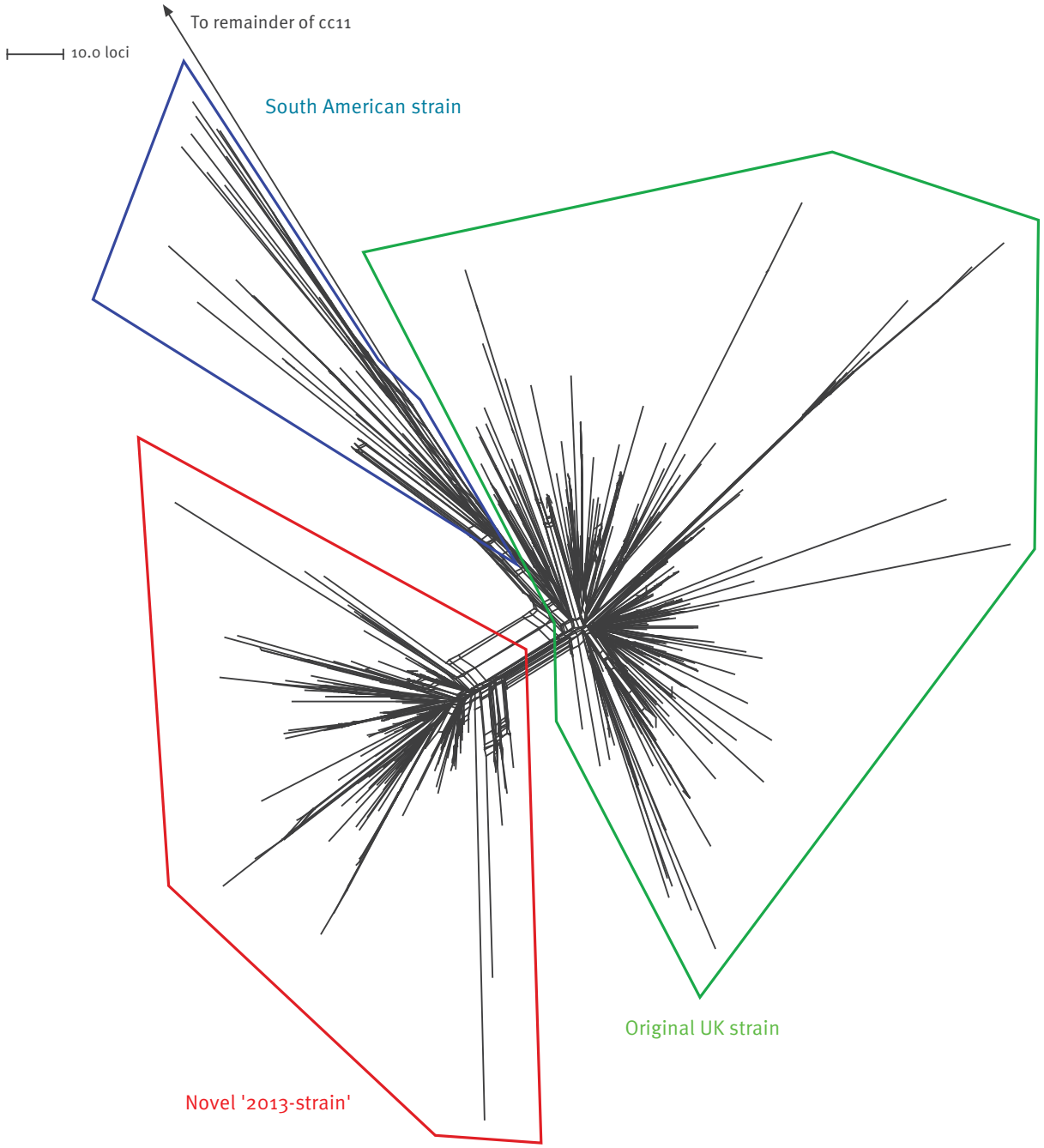
Introduction

Neisseria meningitidis is a leading cause of meningitis and septicaemia [1]. Occurrences of invasive meningococcal disease (IMD) range from sporadic cases to large outbreaks and epidemics. Outbreaks have been associated with mass gatherings such as that of the annual Hajj pilgrimage to Mecca [2]. The 23rd World Scout Jamboree took place between 28 July and 8 August 2015 in Japan and included 33,000 scouts from 162 countries [3]. Over the nine days that followed, three scouts and one non-attending close contact of a healthy scout from the North of Scotland Unit, and two scouts from the Stockholm Unit (Sweden), fell ill with laboratory-confirmed IMD.

All of the patients were admitted to hospital. One of the cases presented with meningitis and shock and was treated in intensive care for six days. The remaining five cases exhibited relatively mild non-specific and/or atypical (respiratory) symptoms. All cases eventually recovered well with no apparent sequelae [3]. A further seven suspected cases among attendees (five in Scotland and two in Sweden, the latter of which represented two further distinct scout units) were eventually discounted. In the course of the outbreak management, chemoprophylaxis was administered to 53 Scottish scouts, leaders and close contacts, and a further individual outside of Scotland. All of them were also offered quadrivalent ACWY conjugate vaccine [4]. In Sweden, where the outbreak initially appeared more diffuse, chemoprophylaxis was offered to all 1,900 Jamboree participants, with an uptake of ca 80% (data not shown). This was accompanied by throat swabbing

Figure 1

Population structure of the South American W:cc11 strain sublineage



cc: clonal complex; UK: United Kingdom.

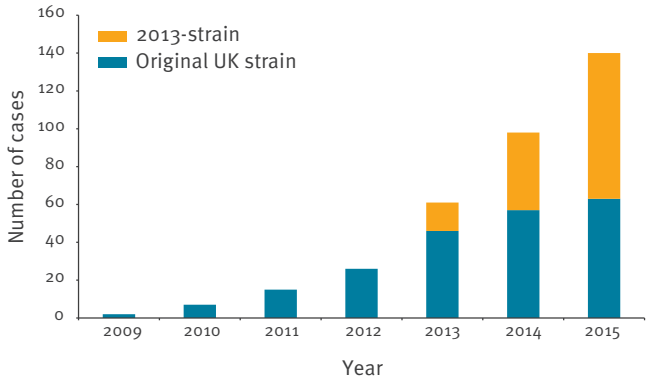
Neighbour-net phylogenetic network based on a comparison of 1,546 core genome loci among all South American W:cc11 strain sublineage genomes (n=454; accessed on 21 January 2016) on the PubMLST database. A single serogroup B lineage 11.2 genome (M09 240026) was used to represent the remainder of cc11 ('to remainder of cc11'). The sublineage was divided into three main strains, the South American strain, the original United Kingdom (UK) strain that emerged in the UK in 2009, and the novel '2013-strain' that emerged in the UK in 2013. The scale bar indicates the number of differences among the 1,546 loci compared.

to assess the meningococcal carriage rate during the outbreak.

Invasive meningococcal isolates (from sterile sites) were obtained from each of the Scottish cases and one of the Swedish cases. The other Swedish case yielded a throat swab isolate and was confirmed as a case serologically by a complement binding assay exhibiting cross-reactivity against *N. meningitidis* and *N. gonorrhoeae*. The six meningococcal isolates were indistinguishable in terms of serogroup and PorA subtype (serogroup W, PorA subtype P1.5,2,36–2). The four

Figure 2

Cases of culture-confirmed invasive meningococcal disease caused by the original United Kingdom strain and 2013-strain of the South American W:cc11 strain sublineage, by year, England, Wales and Northern Ireland, 2009–2015 (n = 349)



cc: clonal complex; UK: United Kingdom.

The chart includes five isolates from pre-Meningococcus Genome Library (2009: 2 and 2010: 3) and includes all corresponding isolates received by the Meningococcal Reference Unit up to 4 November 2015.

Scottish isolates also underwent FetA and multilocus sequence typing (MLST) and, again, were identical to one another (FetA F1–1 and sequence type (ST)-11) [3]. ST-11 is part of the ST-11 clonal complex (cc11) which is associated with multiple serogroups, a tendency to cause outbreaks and epidemics, atypical clinical presentations, and relatively high case fatality rates [5]. Serogroup C cc11, for example, has caused outbreaks among military recruits [6], university undergraduates [7] and more recently, men who have sex with men [8]. Serogroup W cc11 (W:cc11), meanwhile, was responsible for the global Hajj-associated outbreak in 2000 [2], followed by several large epidemics in sub-Saharan Africa recently reviewed by Mustapha et al. [9], and the expansion of endemic disease in South Africa [10], South America [11] and Europe [12].

As with the recent scout cases, the vast majority of W:cc11 isolates from each of the above episodes are indistinguishable using routine typing schemes (up to and including the level of MLST) [13]. As a consequence, the organisms responsible have collectively been described as the 'Hajj strain', denoting the first large outbreak characterised as such. Relatively high-resolution techniques such as pulsed-field gel electrophoresis, however, were indicative of underlying diversity [14]. More recently, genome-level comparisons have indicated that almost all W:cc11 isolates belong to cc11 lineage 11.1, one of two divergent cc11 lineages. Furthermore, W:cc11 isolates corresponding to the major W:cc11 outbreaks were resolved into distinct clusters (strains) within two divergent lineage 11.1 sublineages [13]. The Hajj strain sublineage comprises the W:cc11 Hajj outbreak strain, sub-Saharan African W:cc11 strains from epidemic periods, and the recent endemic South African W:cc11 strain. The South American W:cc11 strain sublineage (previously designated the 'South American/United Kingdom (UK) strain') charts the diversification of the South American strain and closely related UK strain during expansion from southern Brazil, through Argentina and Chile and onto the UK and Europe. Each distinct strain represents clonal expansion from a single ancestor and may be defined by the genetic differences that distinguish it from closely related strains.

The current study sought to determine (i) which of the Jamboree-associated cases represented a genuine outbreak, (ii) to identify the strain/s responsible and its/their relationship to other geo-temporally diverse W:cc11 isolates, (iii) to chart its/their carriage among the Swedish returnees, and (iv) to identify its defining genomic characteristics.

Methods

Genomes

The study used all W:cc11 genomes on the PubMLST *Neisseria* database [15] (n = 873; accessed 21/01/16). These included the Scottish (n = 4) and Swedish (n = 2) outbreak isolates and carrier isolates from Swedish Jamboree attendees (n = 10). The latter 16 isolates are hereafter referred to as the 'Jamboree-associated' isolates. The W:cc11 panel also included genomes from earlier Scottish (2015, n = 11; 2013, n = 1; and 2012, n = 1) and Swedish (2015, n = 6) cases. A separate subset of sero/genogroup B, C and W cc11 genomes were used as a representative panel spanning the known diversity of cc11 (n = 106; Box) [13].

Genomic analyses

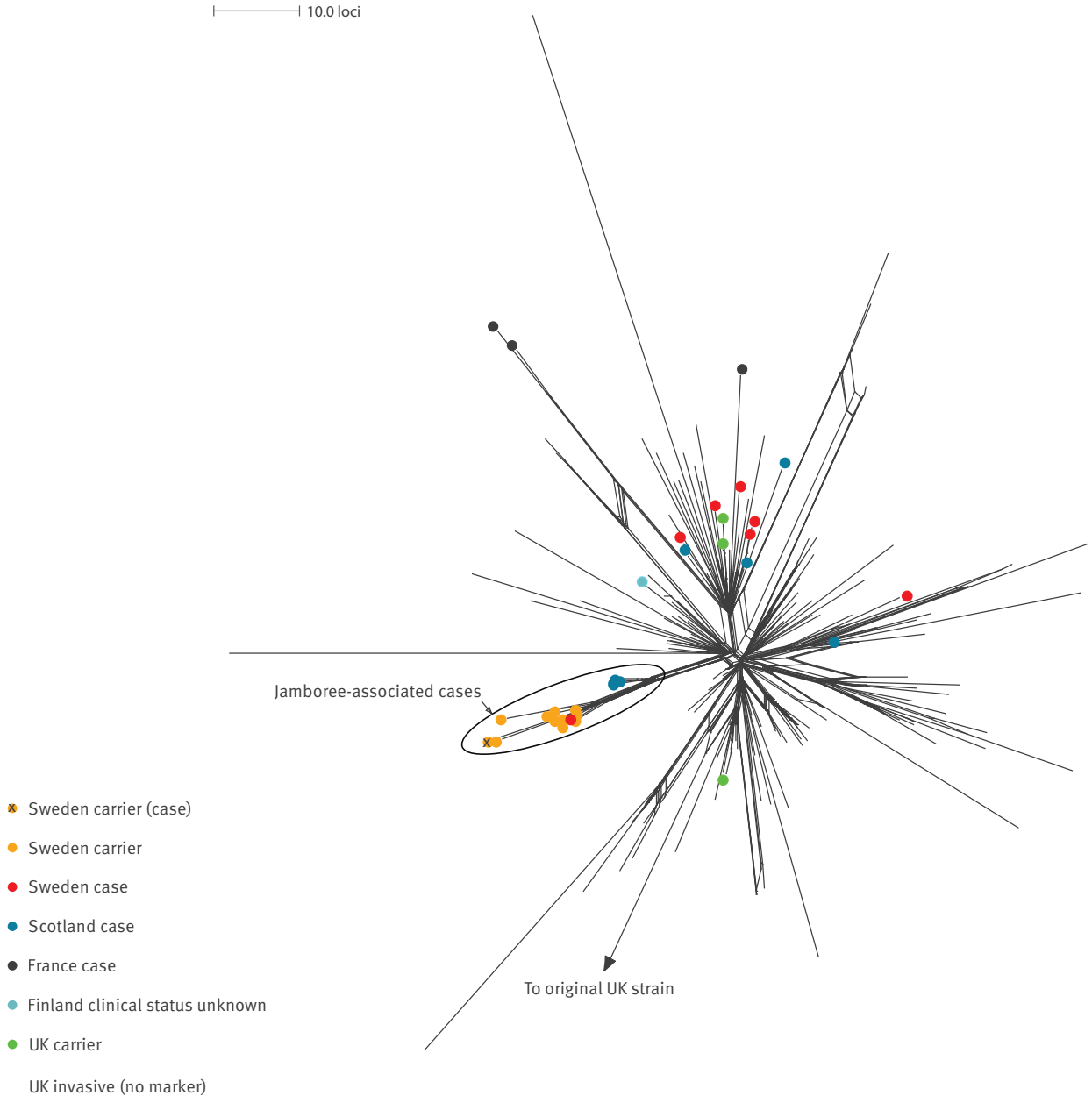
Genome comparisons were performed using the PubMLST genome comparator tool [16]. In order to map their diversity on a 'macro' scale, all of the W:cc11 genomes (n = 873) were initially split into two manageable groups and each group, along with the representative panel spanning the known diversity of cc11, underwent genome comparisons in terms of every 50th core gene (numerically) starting with BACT000001. Refined analyses of the population comprising the Jamboree-associated and related genomes were performed using 1,546 core genome loci [12]. Genetic differences defining the Jamboree-associated and related genomes were identified by comparing these and related genomes in terms of all corresponding indexed 'neis' loci on the PubMLST *Neisseria* database. Resulting distance matrices were visualised using SplitsTree4 [17].

Results

In initial comparisons (using 52 core genes) including a panel of isolates representing the known diversity of cc11, the Jamboree-associated isolates were found

Figure 3

Population structure and geographical distribution of isolates belonging to the 2013-strain of the South American W:cc11 strain sublineage



cc: clonal complex; UK: United Kingdom.

Neighbour-net phylogenetic network based on a comparison of all corresponding indexed 'neis' loci among all W:cc11 2013-strain genomes (n = 169) on the PubMLST Neisseria database. A single original United Kingdom (UK) strain genome (M14 240001) was used to represent the original UK strain (To original UK strain). The Jamboree-associated isolates (four Scottish cases, two Swedish cases and 10 Swedish carriers) belonged to a distinct cluster – the Jamboree-associated cluster. The other isolates included case isolates from the UK (n = 144; unmarked), France (n = 3) and Sweden (n = 6), carrier isolates from the UK (n = 3), and a Finnish isolate of unknown status. The scale bar indicates the number of differences among all corresponding indexed 'neis' loci.

to cluster with isolates of the South American W:cc11 strain sublineage (data not shown).

Box

PubMLST Neisseria IDs of a panel of serogroup B, C and W cc11 genomes spanning the known diversity of cc11

19957, 29677, 29680, 29681, 29683, 21573, 21578, 21582, 21583, 21584, 30087, 30088, 30089, 30090, 30092, 27087, 29679, 29705, 30076, 30077, 19968, 20057, 20154, 20158, 20196, 29633, 29580, 27089, 20066, 29631, 29976, 29664, 21134, 21311, 21330, 26824, 27803, 26733, 26821, 29639, 28103, 21208, 30295, 29571, 30060, 29908, 30284, 29789, 1170, 29590, 29641, 644, 30296, 30244, 29840, 29849, 29858, 29865, 314, 30239, 30240, 30241, 30243, 344, 29611, 29626, 29638, 29891, 21335, 29578, 21196, 29643, 665, 20261, 29831, 30257, 30261, 30260, 21587, 29315, 29329, 29330, 29381, 29324, 29325, 29328, 29331, 29349, 21581, 29334, 29340, 29341, 29366, 29648, 29649, 29651, 29652, 29653, 29709, 29710, 30178, 30234, 30237, 29704, 30183, 30184

Panel selected from [13].

A core genome comparison (1,546 loci) of all of the South American W:cc11 strain sublineage genomes revealed the existence of a novel strain alongside the previously described South American strain and original UK strain that emerged in 2009 [13] (Figure 1).

The novel '2013-strain' emerged in the UK in 2013 and included all of the Jamboree-associated isolates as well as additional invasive isolates from the UK (2013–2015: 144), France (2015: 3) and Sweden (2015: 6). It also included three UK carrier isolates and a single Finnish isolate (2015) of unknown clinical status. UK cases due to the 2013-strain have approximately doubled year-on-year since its emergence while the initially comparable rate of expansion of the original UK strain began to slow (Figure 2).

The 2013-strain isolates and a single original UK strain isolate (M14 240001) underwent a comprehensive genome comparison in terms of all corresponding indexed 'neis' loci on the PubMLST *Neisseria* database. The Jamboree-associated isolates exclusively formed a distinct cluster within the 2013-strain (Figure 3).

Within the 'Jamboree-associated cluster' the Scottish case isolates and Swedish case/carrier isolates formed separate subclusters relatively close/distant to the origin of the main cluster, respectively.

All isolates belonging to the original UK and 2013-strains were compared in terms of all corresponding indexed 'neis' loci, as above. The genome comparator output data were examined for common differences distinguishing the 2013-strain from the original UK strain. The transition included three putative recombination events involving 17, six and two genes, respectively, and four point mutations (Table).

Genes affected included those encoding antigens (including the haemoglobin-haptoglobin receptor complex HpuAB), the genetic regulator MtrR, and a number of housekeeping/metabolic genes. In addition, the predominant *csw* (serogroup W determinant) genes of the respective strains differed by two compensatory frameshift mutations. As one of these was in a homopolymer, it is uncertain whether these constituted spontaneous mutations or a small recombination event.

Allelic variants within the largest recombinant region (nmb0813 to 0829) ranged from being unique to the W:cc11 isolates (neis0813, neis0815, neis0819, neis0825 and neis0827 to 8) to being observed among isolates belonging to various ccs on the PubMLST database. BLAST searches on the NCBI nt database failed to identify exact matches for the unique W:cc11 alleles. Four out of six alleles within the second largest recombinant region (neis1131 to neis1136) were also observed among multiple ccs. Of the remaining two, neis1131 was unique to the W:cc11 isolates. Only three non-cc11 isolates within the database matched all five of the non-unique alleles – isolate IDs 40007 and 40393 (both ST-10144; 1 invasive and 1 not specified) and isolate ID 20026 (ST-9880; invasive). The acquired *hpuA* and *hpuB* alleles were novel on both the PubMLST and nt databases.

Discussion

High resolution genomic analyses indicated that the Scottish and Swedish IMD cases associated with the 23rd World Scout Jamboree constituted a genuine outbreak with transmission of meningococci belonging to a distinct phylogenetic cluster over a short period of time.

Isolates from Scottish cases were relatively closely related to one-another, probably reflecting prolonged intragroup contact. Isolates from the Swedish cases and carriers were similarly grouped but at a more distal location within the overall cluster, probably reflecting the spread of carriage among the wider Jamboree participants and further group-wise propagation. Broader dissemination of organisms belonging to the cluster was evident from the Scottish case that occurred in a non-attending close contact. None of the 62 W:cc11 submissions made to the PubMLST *Neisseria* database subsequent to the outbreak, including post-Jamboree cases from Sweden (2015: 2 and 2016: 7), the UK (2015: 33), and France (2016: 11), have, however, belonged to the Jamboree-associated cluster (accessed 20 April 2016; data not shown). Ongoing and retrospective genomic surveillance will determine whether the public health interventions employed in the respective countries have served to curtail onward transmission of organisms belonging to the outbreak cluster.

The Jamboree-associated cluster formed part of a novel strain, the proposed '2013-strain', which emerged in the UK in 2013, with cases approximately doubling annually. This strain represented clonal expansion from a single descendant, or close relative, of the original UK strain which emerged in England in 2009 exhibiting

Table

Common genetic differences distinguishing the 2013-strain from the original UK strain (grouped into putative recombinations where appropriate)

Gene[a]	MC58 identifier[b]	Gene product	Impact[c]
NEIS0813	NMB0872	Putative periplasmic protein	None
NEIS0814	NMB0873	Outer membrane lipoprotein LolB	1 aa change
NEIS0815	NMB0874	4-diphosphocytidyl-2-C-methyl-D-erythritol kinase	16 aa changes
NEIS0816	NMB0875	Ribose-phosphate pyrophosphokinase	None
NEIS0817	NMB0876	50S ribosomal protein L25 (*rplY*)	None
NEIS0818	NMB0877	Putative D-alanyl-D-alanine carboxypeptidase	5 aa changes
NEIS0819	NMB0878	Threonine dehydratase	2 aa changes
NEIS0820	NMB0879	Putative sulphate permease ATP-binding protein	1 aa change
NEIS0821	NMB0880	Putative sulphate permease inner membrane protein	2 aa changes
NEIS0822	NMB0881	Sulphate permease inner membrane protein (*cysU*)	1 aa change
NEIS0823	NMB0882	Hypothetical protein	1 aa change
NEIS0824	NMB0883	Hypothetical protein	2 aa changes
NEIS0825	NMB0884	Superoxide dismutase (*sodB*)	2 aa changes
NEIS0826	NMB0885	Replicative DNA helicase	2 aa changes
NEIS0827	NMB0886	Type IV biogenesis protein (*pilH*)	5 aa changes
NEIS0828	NMB0887	Type IV biogenesis protein (*pilI*)	1 aa change
NEIS0829	NMB0888	Type IV biogenesis protein (*pilJ*)	2 aa changes
NEIS1131	NMB1231	Putative ATP-dependent protease	1 aa change
NEIS1132	NMB1232	Hypothetical protein	In-frame gene acquired[d]
NEIS1133	NMB1233	Exodeoxyribonuclease V alpha subunit	11 aa changes
NEIS1134	NMB1234	Putative ABC-transporter ATP-binding protein	7 aa changes
NEIS1135	NMB1235	Putative integral membrane protein	4 aa changes
NEIS1136	NMB1236	Hypothetical protein	None
NEIS1351	NMB1418	Lipid A biosynthesis lauroyl acyltransferase (lpxL)	1 aa change[e]
NEIS1386	NMB1448	DNA polymerase IV	1 aa change[e]
NEIS1412	NMB1475	Hypothetical protein	1 aa change[e]
NEIS1635	NMB1717	Transcriptional regulator (*mtrR*)	Frameshift[f]
NEIS1946	NA	Haemoglobin-haptoglobin utilisation protein (*hpuA*)	25 aa changes[g]
NEIS1947	NA	Haemoglobin-haptoglobin utilisation protein (*hpuB*)	44 aa changes
NEIS2162	NA	Glycosyltransferase (*csw*)	2 aa changes[h]

NA: not applicable; UK: United Kingdom.

[a] PubMLST *Neisseria* database identifier.

[b] MC58 strain identifier, GenBank accession number AE002098.2.

[c] Regarding predominant alleles for respective strains.

[d] Gene frameshifted in original UK strain.

[e] Single nt polymorphism.

[f] Single bp insertion.

[g] If homopolymer normalised and within frame.

[h] Two existing alleles; two compensatory frameshifts.

(initially) a similar rate of expansion. The expansion of serogroup W disease in Scotland became evident from 2014 [18], however, the present study identified earlier cases caused by the original UK strain (2012: 1) and 2013-strain (2013: 1), respectively. Prior to the case in 2012, Scotland experienced no W:cc11 cases for at least three years. After 2013, endemic Scottish W:cc11 cases were distributed among both strains. Sweden experienced a greater than three-fold rise in W:cc11 cases in 2015 mainly due to the 2013-strain (n = 7), with a single additional isolate from the Hajj strain subline-age. The 2013-strain was also responsible for the only two Swedish W:cc11 cases in 2014 (data not shown). Prior to 2014, Sweden experienced one confirmed W:cc11 case per year dating back to 2010 (correspond-ing strains unknown).

Despite cc11 having been associated with numerous focal outbreaks in the past [2,6,7], to our knowledge the original UK strain has only been associated with a single focal outbreak, namely two cases in a healthcare setting in the UK [12,19], with a further four suspected UK outbreaks discounted using the methods described herein (data not shown). The rapid expansion of

2013-strain cases and a possible association with outbreaks may represent heightened carriage, transmission, invasiveness or virulence of the novel strain, or indeed a combination of these factors. This change in epidemiology may, in turn, be a direct consequence of the genetic changes that define the strain. Studies of previous outbreaks/expansions have implicated antigenic shifts in prominent antigens such as PorA [20,21] or fHbp [22], owing to recombination events. As such, possible candidates among 29 altered genes within the 2013-strain include *hpuA* and *hpuB* (encoding the haemoglobin-haptoglobin receptor, HpuAB [23]) which underwent the greatest number of amino acid changes (25 and 44, respectively). Genes involved in the surface expression of other proteins may also be implicated such as the three genes involved in type IV pilus biogenesis (neis0827–8) [24]. Indeed, haemoglobin receptors and pili are not only major antigens but also important virulence factors. Interestingly, relatively remote genes involved in the translocation of lipoproteins to the outer membrane (neis0814, neis0815 and neis1134) were affected by two of the three putative recombination events.

The acquisition of a frameshifted *mtrR* allele may be significant. MtrR is a transcriptional regulator concerned with the expression of various genes in *N. gonorrhoeae*, including those encoding multidrug efflux pumps and others involved in stress responses [25]. It has also been proposed that mutant (including frameshifted) *mtrR* alleles may be advantageous for *N. gonorrhoeae* during infection [25]. MtrR has also recently been implicated in the regulation of *nadA* expression in the meningococcus. NadA is a major surface antigen and a virulence factor involved in adherence and invasion [26,27]. It is also a component of the multicomponent vaccine developed to target serogroup B meningococci (Bexsero) and the likely target of corresponding protection that has been demonstrated against isolates of the original UK W:cc11 strain [28].

The involvement of the *lpxL* gene is noteworthy because this gene is involved in acylation of endotoxin. Frameshifts in *lpxL* have, for example, been implicated in milder disease [29] which was a feature among the Scottish cases but not the Swedish cases, one of whom required six days of intensive care [3]. In the course of routine serogrouping, no obvious effect was observed for the altered *csw* gene. The Public Health England serogrouping assay [30] would not, however, be expected to identify subtle qualitative/quantitative differences in capsule composition.

We were unable to identify potential donor strains involved in several of the putative recombinations and those that we did identify did not belong to common invasive lineages among countries regularly submitting genomic data to the NCBI nt or PubMLST *Neisseria* databases. Other *Neisseria* species less well represented on the sequence databases also constitute potential donors [31]. The acquisition of relatively rare alleles, especially those relating to surface antigens may be advantageous owing to the naivety of the human host population. Genomic analyses of recent carriage studies may shed further light on the identity of the respective donor strains [32,33].

The exact cause of the expansion of the 2013-strain may never be known. Indeed, it may be that this strain has by chance encountered several environments conducive to widespread transmission, such as universities and mass gatherings. Nonetheless, the current analyses revealed that that the continued expansion of W:cc11 in the UK is largely due to the 2013-strain while the expansion of the original UK strain appears to have slowed. Whether the 2013-strain is destined to follow a similar course may also not be known since it is hoped that the recent introduction of the quadrivalent ACWY conjugate vaccine to UK adolescents, including new university entrants, will lead to wider herd protection [34]. Within the 2013-strain, the appearance of the Jamboree-associated cluster appears to have been transient. Should it re-emerge to expand in a way that is comparable to either the 2013- or original UK strains then further investigation may be warranted to identify its defining genetic changes.

The present study demonstrates the utilisation of genomic analysis, in conjunction with comprehensive geo-temporally diverse genomes, to identify bacterial outbreak strains within highly clonal populations. It also demonstrates how such studies may shed light on the emergence of outbreak strains, inform immunisation policy, and, perhaps, inform the development of new vaccines and even therapeutics.

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Conflict of interest

None declared.

Authors' contributions

JL performed data analyses and wrote the manuscript. KS, RU, DL, BS, SJ and SJG performed laboratory work and data analyses. CC, ASP, JM, HC and SL performed data analyses. KS, RU, AS, DL, BS, SJ, HF, CC, ASP, JM, SJG, HC, SL, JF, PM and RB reviewed and contributed to the manuscript. AS, PM and RB coordinated the study.

References

1. Halperin SA, Bettinger JA, Greenwood B, Harrison LH, Jelfs J, Ladhani SN, et al. The changing and dynamic epidemiology of meningococcal disease. Vaccine. 2012;30(Suppl 2):B26-36. DOI: 10.1016/j.vaccine.2011.12.032 PMID: 22178525
2. Taha MK, Achtman M, Alonso JM, Greenwood B, Ramsay M, Fox A, et al. Serogroup W135 meningococcal disease in Hajj pilgrims. Lancet. 2000;356(9248):2159. DOI: 10.1016/S0140-6736(00)03502-9 PMID: 11191548
3. Smith-Palmer A, Oats K, Webster D, Taylor S, Scott K, Smith G, et al. Outbreak of Neisseria meningitidis capsular group W among Scouts returning from the World Scout Jamboree, Japan, 2015. Euro Surveill. 2016;21(45):22636.
4. Health Protection Agency (HPA). Guidance for public health management of meningococcal disease in the UK. London: HPA. 2012. Available from: https://www.gov.uk/government/publications/meningococcal-disease-guidance-on-public-health-management
5. Campbell H, Parikh SR, Borrow R, Kaczmarski E, Ramsay ME, Ladhani SN. Presentation with gastrointestinal symptoms and high case fatality associated with group W meningococcal disease (MenW) in teenagers, England, July 2015 to January 2016.Euro Surveill. 2016;21(12):30175. DOI: 10.2807/1560-7917.ES.2016.21.12.30175 PMID: 27035055
6. Brundage JF, Zollinger WD. Evolution of meningococcal disease epidemiology in the US army. In: Vedros NA, editor. Evolution of meningococcal disease. Boca Raton: CRC Press; 1987.
7. Clusters of meningococcal disease in university students. Commun Dis Rep CDR. Wkly. 1997 Oct 31;7(44):393, 396.
8. Kupferschmidt K. Infectious diseases. Bacterial meningitis finds new niche in gay communities.Science. 2013;341(6144):328. DOI: 10.1126/science.341.6144.328 PMID: 23888010
9. Mustapha MM, Marsh JW, Harrison LH. Global epidemiology of capsular group W meningococcal disease (1970-2015): Multifocal emergence and persistence of hypervirulent sequence type (ST)-11 clonal complex.Vaccine. 2016;34(13):1515-23. DOI: 10.1016/j.vaccine.2016.02.014 PMID: 26876439
10. von Gottberg A, du Plessis M, Cohen C, Prentice E, Schrag S, de Gouveia L, et al. , Group for Enteric, Respiratory and Meningeal Disease Surveillance in South Africa. Emergence of endemic serogroup W135 meningococcal disease associated with a high mortality rate in South Africa.Clin Infect Dis. 2008;46(3):377-86. DOI: 10.1086/525260 PMID: 18181736
11. Abad R, López EL, Debbag R, Vázquez JA. Serogroup W meningococcal disease: global spread and current affect on the Southern Cone in Latin America.Epidemiol Infect. 2014;142(12):2461-70. DOI: 10.1017/S0950268814001149 PMID: 24831052
12. Ladhani SN, Beebeejaun K, Lucidarme J, Campbell H, Gray S, Kaczmarski E, et al. Increase in endemic Neisseria meningitidis capsular group W sequence type 11 complex associated with severe invasive disease in England and Wales. Clin Infect Dis. 2015;60(4):578-85. DOI: 10.1093/cid/ciu881 PMID: 25389259
13. Lucidarme J, Hill DM, Bratcher HB, Gray SJ, du Plessis M, Tsang RS, et al. Genomic resolution of an aggressive, widespread, diverse and expanding meningococcal serogroup B, C and W lineage. J Infect. 2015;71(5):544-52. DOI: 10.1016/j.jinf.2015.07.007 PMID: 26226598
14. Mayer LW, Reeves MW, Al-Hamdan N, Sacchi CT, Taha MK, Ajello GW, et al. Outbreak of W135 meningococcal disease in 2000: not emergence of a new W135 strain but clonal expansion within the electophoretic type-37 complex. J Infect Dis. 2002;185(11):1596-605. DOI: 10.1086/340414 PMID: 12023765
15. PubMLST Neisseria Sequence Typing Home Page. Oxford: PubMLST. [Accessed 21 January 2016]. Available from: http://pubmlst.org/neisseria
16. Bratcher HB, Corton C, Jolley KA, Parkhill J, Maiden MC. A gene-by-gene population genomics platform: de novo assembly, annotation and genealogical analysis of 108 representative Neisseria meningitidis genomes.BMC Genomics. 2014;15(1):1138. DOI: 10.1186/1471-2164-15-1138 PMID: 25523208
17. Huson DH. SplitsTree: analyzing and visualizing evolutionary data.Bioinformatics. 1998;14(1):68-73. DOI: 10.1093/bioinformatics/14.1.68 PMID: 9520503
18. Health Protection Scotland (HPS). Respiratory bacteria quarterly report. Quarter four: 1 October to 31 December 2015. HPS Weekly Report. 2016 Mar 22;50(2016/12):87-90. Available from: http://www.hps.scot.nhs.uk/documents/ewr/pdf2016/1612.pdf
19. Puleston R, Beck C, Tahir M, Bardhan M, Charlemagne P, Alves C, et al. An unusual transmission event of Neisseria meningitidis serogroup W135 type 2a in a healthcare setting, England, 2012. Euro Surveill. 2012;17(44):20308.PMID: 23137486
20. Harrison LH, Jolley KA, Shutt KA, Marsh JW, O'Leary M, Sanza LT, et al. , Maryland Emerging Infections Program. Antigenic shift and increased incidence of meningococcal disease.J Infect Dis. 2006;193(9):1266-74. DOI: 10.1086/501371 PMID: 16586364
21. Tsang RS, Law DK, Henderson AM, Blake ML, Stoltz J. Increase in serogroup C meningococcal disease in Canada is associated with antigenic changes in the protein antigens of the ET-15 clone of Neisseria meningitidis.J Infect Dis. 2006;194(12):1791-2, author reply 1792-3. DOI: 10.1086/509515 PMID: 17109354
22. Mustapha MM, Marsh JW, Krauland MG, Fernandez JO, de Lemos AP, Dunning Hotopp JC, et al. Genomic Epidemiology of Hypervirulent Serogroup W, ST-11 Neisseria meningitidis. EBioMedicine. 2015;2(10):1447-55. DOI: 10.1016/j.ebiom.2015.09.007 PMID: 26629539
23. Tauseef I, Harrison OB, Wooldridge KG, Feavers IM, Neal KR, Gray SJ, et al. Influence of the combination and phase variation status of the haemoglobin receptors HmbR and HpuAB on meningococcal virulence. Microbiology. 2011;157(Pt 5):1446-56. DOI: 10.1099/mic.0.046946-0 PMID: 21310784
24. Berry JL, Pelicic V. Exceptionally widespread nanomachines composed of type IV pilins: the prokaryotic Swiss Army knives. FEMS Microbiol Rev. 2015;39(1):134-54. DOI: 10.1093/femsre/fuu001 PMID: 25793961
25. Folster JP, Johnson PJ, Jackson L, Dhulipali V, Dyer DW, Shafer WM. MtrR modulates rpoH expression and levels of antimicrobial resistance in Neisseria gonorrhoeae.J Bacteriol. 2009;191(1):287-97. DOI: 10.1128/JB.01165-08 PMID: 18978065
26. Cloward JM, Shafer WM. MtrR control of a transcriptional regulatory pathway in Neisseria meningitidis that influences expression of a gene (nadA) encoding a vaccine candidate.PLoS One. 2013;8(2):e56097. DOI: 10.1371/journal.pone.0056097 PMID: 23409129
27. Nägele V, Heesemann J, Schielke S, Jiménez-Soto LF, Kurzai O, Ackermann N. Neisseria meningitidis adhesin NadA targets beta1 integrins: functional similarity to Yersinia invasin.J Biol Chem. 2011;286(23):20536-46. DOI: 10.1074/jbc.M110.188326 PMID: 21471204
28. Ladhani SN, Giuliani MM, Biolchi A, Pizza M, Beebeejaun K, Lucidarme J, et al. Effectiveness of Meningococcal B Vaccine against Endemic Hypervirulent Neisseria meningitidis W Strain, England. Emerg Infect Dis. 2016;22(2):309-11. DOI: 10.3201/eid2202.150369 PMID: 26811872
29. Brouwer MC, Spanjaard L, Prins JM, van der Ley P, van de Beek D, van der Ende A. Association of chronic meningococcemia with infection by meningococci with underacylated lipopolysaccharide.J Infect. 2011;62(6):479-83. DOI: 10.1016/j.jinf.2011.03.010 PMID: 21459106
30. Gray SJ, Trotter CL, Ramsay ME, Guiver M, Fox AJ, Borrow R, et al. , Meningococcal Reference Unit. Epidemiology of meningococcal disease in England and Wales 1993/94 to 2003/04: contribution and experiences of the Meningococcal Reference Unit.J Med Microbiol. 2006;55(Pt 7):887-96. DOI: 10.1099/jmm.0.46288-0 PMID: 16772416
31. Wörmann ME, Horien CL, Bennett JS, Jolley KA, Maiden MC, Tang CM, et al. Sequence, distribution and chromosomal context of class I and class II pilin genes of Neisseria meningitidis identified in whole genome sequences. BMC Genomics. 2014;15(1):253. DOI: 10.1186/1471-2164-15-253 PMID: 24690385
32. Read RC, Baxter D, Chadwick DR, Faust SN, Finn A, Gordon SB, et al. Effect of a quadrivalent meningococcal ACWY glycoconjugate or a serogroup B meningococcal vaccine on meningococcal carriage: an observer-blind, phase 3 randomised clinical trial. Lancet. 2014;384(9960):2123-31. DOI: 10.1016/S0140-6736(14)60842-4 PMID: 25145775
33. MacLennan J, Maiden MCUK. _Meningococcocal_carriage_ Group. UKMENCAR4: A meningococcal carriage study in 21,000 teenagers to understand changing meningococcal epidemiology and evaluate national vaccination policy. 13th EMGM. Amsterdam, The Netherlands; 2015.
34. Campbell H, Saliba V, Borrow R, Ramsay M, Ladhani SN. Targeted vaccination of teenagers following continued rapid endemic expansion of a single meningococcal group W clone (sequence type 11 clonal complex), United Kingdom 2015. Euro Surveill. 2015;20(28):21188. DOI: 10.2807/1560-7917.ES2015.20.28.21188 PMID: 26212140

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Research article

Factors influencing the spread of pertussis in households: a prospective study, Catalonia and Navarre, Spain, 2012 to 2013

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We aimed to investigate transmission rates of pertussis in household contacts of cases and factors associated with transmission. A prospective epidemiological study was conducted in 2012 and 2013 to determine the incidence of pertussis among household contacts of reported cases in Catalonia and Navarre, Spain. An epidemiological survey was completed for each case and contact, who were followed for 28 days to determine the source of infection (primary case) and detect the occurrence of secondary cases. Odds ratios (ORs) were used to estimate the effectiveness of vaccination and chemoprophylaxis in preventing new cases, using the formula (1 – OR) × 100. For the 688 primary cases, a total of 2,852 contacts were recorded. The household transmission rate was 16.1% (459/2,852) and rose according to the age (>18 years) and lack of immunisation of the primary cases, and also the age (0–18 years), family relationship (siblings and children), lack of vaccination and chemoprophylaxis of contacts. Pertussis vaccine effectiveness in preventing new cases was 65.0% (95% confidence interval (CI): 11.6 to 86.2) for full vaccination (≥ 4 doses) and 59.7% (95% CI: –6.8 to 84.8) for incomplete vaccination (< 4 doses). The effectiveness of chemoprophylaxis was 62.1% (95% CI: 40.3 to 75.9). To reduce household transmission, contacts should be investigated to detect further cases and to administer chemoprophylaxis. The current vaccination status of cases and contacts can reduce household transmission.

Introduction

Pertussis vaccination has led to an important reduction in the incidence of the disease in children in the past 60 years [1]. However, pertussis remains a vaccine-preventable disease that causes a large number of deaths worldwide [2] and has high incidence and hospitalisation rates, even in industrialised countries [3,4].

Studies suggest that the persistence of transmission of the causative agent, *Bordetella pertussis*, is due to the fact that immunity to *B. pertussis* infection – whether acquired naturally or by vaccination – is not lifelong [5,6]. In fact, a second infection in people who have already been infected with *B. pertussis* have been reported [7]. When whole-cell vaccines (wPs) are used, protective antibodies decline by 50% over a period of 6 to 12 years [5,8]. The duration of immunity conferred by acellular vaccines (aPs) – which are used today in most industrialised countries because they are less reactogenic [9] – appears to be shorter than that conferred by wP [10,11]. Some studies suggest that aPs induce a suboptimal immune response that is unable to prevent infection, thus providing a plausible explanation for pertussis resurgence [12].

In Spain, the wP against pertussis, combined with diphtheria and tetanus toxoids (DTwP), was commercialised in the 1960s and was administered to infants (aged under 1 year) in two annual campaigns [13]. In Catalonia and Navarre, the wP was included in 1980

Table 1

Characteristics of primary cases of pertussis with household contacts, Catalonia and Navarre, Spain, 2012–13 (n = 688)

Characteristic of primary case	Number	%
Sex		
Male	325	47.2
Female	363	52.8
Age in years		
‹1	151	21.9
1	24	3.5
2–3	44	6.4
4–6	76	11.0
7–10	149	21.7
11–18	98	14.2
19–40	76	11.0
›40	70	10.2
Clinical symptoms		
Cough lasting ›2 weeks	644	93.6
Paroxysmal cough	581	84.4
Post-tussive vomiting	276	40.1
Inspiratory stridor	259	37.6
Apnoea	151	21.9
Fever	74	10.8
Laboratory confirmation (PCR and/or culture)		
Yes	504	73.3
No	184	26.7
Hospitalisation		
Yes	105	15.3
No	583	84.7
Vaccination status[a]		
Fully vaccinated	331	48.1
Undervaccinated due to age	90	13.1
Undervaccinated	15	2.2
Unvaccinated	61	8.9
Unvaccinated due to age	66	9.6
Unknown/no answer	125	18.2

[a] Vaccination status was categorised as fully vaccinated (≥ 4 doses of vaccine), undervaccinated (‹ 4 doses), unvaccinated (no dose), undervaccinated due to age (‹ 4 doses) and unvaccinated due to age (no dose).

in the national childhood immunisation schedule, with four doses at 3, 5, 7 and 18 months of age. In 1998, the vaccination schedule was changed, reducing the age of administration and number of the wP doses at 2, 4 and 6 months of age, and included two doses of the aP (at 18 months and 4–6 years of age). In 2002, five doses of aP – diphtheria, tetanus and acellular pertussis (DTaP)/ combined tetanus, diphtheria and acellular pertussis (Tdap) – were introduced into the childhood immunisation schedule, with the last dose given at the age of 4–6 years, to reduce the side effects of wP vaccination. In Spain, vaccination coverage with pertussis vaccines has been more than 90% since 1990 [13]. Nevertheless, pertussis incidence increased from less than 1 per 100,000 population in 2003 to 5.3 per 100,000 population in 2013 [13].

Studies of children worldwide hospitalised due to serious outcomes of pertussis have shown that the most frequent source of infection is in the household, due to infection by mothers or other family members (siblings, fathers, grandparents) or caregivers, who presented with symptoms of coughing that were not recognised as being due to pertussis [14-16].

Other studies of community index cases also indicate that *B. pertussis* transmission often occurs in households and that transmission rates in this setting are variable but high, depending on factors related to the pertussis cases and their contacts, such as age, sex or immune status [17,18].

The rate of secondary transmission of *B. pertussis* in Spanish households and the relative importance of family relationships and specific age groups regarding infection is unknown. Similarly, chemoprophylaxis with azithromycin is recommended for post-exposure prophylaxis [19], but its effectiveness, and that of DTwP/DTaP/Tdap vaccination, in preventing transmission in household contacts is also unknown. Such data could be valuable in the assessment of strategies to reduce the number of *B. pertussis* infections, especially in children.

The aim of our study was to investigate the sources of infection of primary cases and rates of secondary transmission of pertussis in contacts of pertussis cases in households and factors associated with transmission in Catalonia and Navarre, Spain.

Methods

A prospective epidemiological study was conducted in 2012 and 2013 on the incidence of pertussis among household contacts of pertussis cases who were reported to the notifiable diseases systems of Catalonia and Navarre, which together have a population of 8.2 million [20].

Index cases (defined below) were reported to public health professionals from the epidemiological surveillance units of the Department of Health of Catalonia, the Public Health Agency of Barcelona and the Public Health Institute of Navarre. Each case notified was considered an index case. To be included in the study, an individual had to meet the criteria for a confirmed case (see below) and have household contacts who could be identified.

For each index case detected, an epidemiological survey of the study variables (outlined below) was completed and household contacts were identified. Each case was asked about exposure to a person with pertussis, symptoms, doses of pertussis vaccine received (registered in an official document or medical history) and preventive measures adopted (vaccination or

Table 2

Characteristics of household contacts of primary cases of pertussis, Catalonia and Navarre, Spain, 2012–13 (n = 2,852)

Characteristic of household contact	Number	%
Sex		
Male	1,340	47.0
Female	1,512	53.0
Age in years		
<1	150	5.3
1	58	2.0
2–3	132	4.6
4–6	200	7.0
7–10	221	7.7
11–18	209	7.3
19–40	967	33.9
>40	915	32.1
Household contacts		
Cohabitant	2,034	71.3
Non-cohabitant	818	28.7
Relationship to primary case		
Mother	556	19.5
Father	510	17.9
Sibling	518	18.2
Grandparent	330	11.6
Child	139	4.9
Partner	100	3.5
Other[a]	699	24.5
Number of contacts in the household		
≤2	226	7.9
3–4	1,133	29.7
>4	1,493	52.3
Vaccination status (≤18 years)[b]		
Fully vaccinated	581	64.4
Undervaccinated due to age	94	10.4
Undervaccinated	27	3.0
Unvaccinated	49	5.4
Unvaccinated due to age	53	5.9
Unknown/no answer	97	10.8
Received chemoprophylaxis[c]		
Yes	2,284	80.1
No	406	14.2
Unknown	162	5.7

[a] Caregiver, family friend or neighbour.

[b] Vaccination status was categorised as fully vaccinated (≥4 doses of vaccine), undervaccinated (<4 doses), unvaccinated (no dose), undervaccinated due to age (<4 doses) and unvaccinated due to age (no dose).

[c] Azithromycin was used.

chemoprophylaxis). As the transmission period of the disease may be as long as 21 days [21] and the incubation period in a new case seven days [22], cases and contacts were followed for 28 days to determine the source of infection (primary case), and the appearance of secondary cases.

Two samples were taken, using appropriate swabs (Dacron or Rayon for PCR) and cotton for cultures), from the posterior nasopharynx of each case and contact with pertussis-compatible symptoms for determining presence of *B. pertussis* by culture or PCR. Swabs for culture were transported in a suitable medium to ensure viability of the bacteria and swabs for PCR were resuspended in 200 µL saline solution. *B. pertussis* DNA was detected using real-time PCR amplification of the insertion sequences *Bordetella* IS481 [23]. Human RnaseP gene was used to check sample quality and detection of inhibitors of PCR reaction.

Definitions

An index case was defined as the first reported pertussis case who generated the study of pertussis in a particular household.

A confirmed case was defined as a person presenting clinically with a cough, together with microbiological confirmation (isolation of *B. pertussis* in culture or positive PCR test from nasopharyngeal swabs) or a person who fulfilled the clinical definition (cough for more than two weeks and at least one of the following: paroxysmal cough, inspiratory stridor, post-tussive vomiting or apnoea) and who was also epidemiologically linked to a confirmed case.

A primary case was defined as the first confirmed case of pertussis in a household to develop symptoms.

A coprimary case was defined a confirmed case of pertussis with symptoms appearing between 0 and 6 days after those of the primary case had started.

A secondary case was a confirmed case in whom symptoms began between 7 and 28 days after those of the primary case.

After completion of the survey and laboratory tests, each index case and household contact was classified as a healthy contact, primary case or secondary case (confirmed microbiologically or by epidemiological link).

Household contacts were defined as all residents of the household of the primary case (cohabitants) or persons who had had contact with the primary case for more than 2 hours (to exclude sporadic contact) in the same dwelling during the transmission period of the disease (non-cohabitants) in order to detect cases among relatives and caregivers who were not household cohabitants but could have a relevant role in the epidemiological chain. We choose 2 hours to eliminate sporadic contact (with less than 2 hours of contact).
The transmission period of the disease was defined as the period of 21 days from the onset of symptoms in the primary case or five days from the onset of treatment of the primary case.

Table 3

Incidence of pertussis in household contacts by characteristic of primary cases (n = 2,852)

Characteristic of primary case	Incidence of pertussis among contacts		Odds ratio	95% CI
	%	n/total		
Sex				
Female	16.0	245/1,528	1.0	0.8 to 1.2
Male	16.2	214/1,324	Reference	
Age in years				
<1	8.9	60/671	Reference	
1	9.6	10/104	1.1	0.5 to 2.2
2–3	10.9	25/229	1.2	0.7 to 2.0
4–6	14.4	47/326	1.7	1.1 to 2.6
7–10	15.3	91/595	1.8	1.3 to 2.6
11–18	14.9	54/363	1.8	1.2 to 2.6
19–40	31.0	90/290	4.6	3.2 to 6.6
>40	29.9	82/274	4.3	3.0 to 6.3
Microbiological confirmation (PCR and/or culture)				
Yes	10.7	219/2,055	Reference	
No	27.0	61/226	3.1	2.2 to 4.3
Unknown	30.8	158/513	3.7	2.9 to 4.7
Hospitalisation				
Yes	10.0	48/479	0.6	0.4 to 0.8
No	17.4	403/2,312	Reference	
Number of contacts				
≤2	19.5	44/226	Reference	
3–4	16.0	181/1,133	0.8	0.6 to 1.1
>4	15.7	234/1,493	0.8	0.6 to 1.1
Vaccination status[a]				
Fully vaccinated	14.1	188/1,331	Reference	
Undervaccinated/ Unvaccinated/Unknown	17.8	271/1,521	1.3	1.1 to 1.6

CI: confidence interval.

[a] Vaccination status was categorised as fully vaccinated (≥4 doses of vaccine), undervaccinated (<4 doses) or unvaccinated (no dose).

Study variables

Information on the following sets of variables was obtained from a face-to-face questionnaire and official records for each pertussis case and each household contact.

Demographic variables: sex, age, number of persons in a household (cohabitant or non-cohabitant) and the relationship between the household members. For contacts, the relationship with the primary case (e.g. mother, father, sibling, grandparent, child, partner, other) was recorded.

Clinical variables: date of onset of first symptom, cough lasting 2 or more weeks, number of days of persistent cough, and presence/absence of paroxysmal coughing, post-tussive vomiting, apnoea, fever, pneumonia, seizures, encephalopathy, hospitalisation.

Laboratory results: type of sample, result of culture and PCR.

Preventive measures: for study participants – all cases of pertussis (all ages) and household contacts (aged ≤18 years) – who had received any dose of pertussis vaccine, the number and date of administration of doses were recorded. The cut-off of 18 years was chosen because few contacts aged more than 18 years had records of their vaccinations. Vaccination status was categorised as fully vaccinated (≥4 doses of vaccine), undervaccinated (<4 doses), unvaccinated (no dose), undervaccinated due to age (<4 doses) and unvaccinated due to age (no dose).

Chemoprophylaxis was defined as completion of antibiotic treatment (azithromycin) in a healthy contact (all ages) initiated after symptom onset of the primary case.

Sample size

Given that the annual median number of new cases in Catalonia and Navarre was 203 [24] and the study

Table 4

Incidence of pertussis in household contacts by characteristic, Catalonia and Navarre, Spain, 2012–13 (n = 2,852)

Characteristic of household contact	Incidence of pertussis among contacts		Odds ratio	95% CI
	%	n/total		
Sex				
Female	15.9	241/1,512	1.0	0.8 to 1.2
Male	16.3	218/1,340	Reference	
Age in years				
<1	69.3	104/150	24.6	16.2 to 37.4
1	44.8	26/58	8.8	5.0 to 15.6
2–3	25.0	33/132	3.6	2.3 to 5.7
4–6	21.5	43/200	3.0	2.0 to 4.5
7–10	19.5	43/221	2.6	1.7 to 3.9
11–18	19.1	40/209	2.6	1.7 to 3.9
19–40	9.6	93/967	1.2	0.8 to 1.6
>40	8.4	77/915	Reference	
Household contacts				
Cohabitants	16.5	336/2034	1.1	0.9 to 1.4
Non-cohabitants	15.0	123/818	Reference	
Relationship with primary case				
Mother	8.3	46/556	1.8	1.0 to 3.4
Father	8.8	45/510	2.0	1.1 to 3.7
Sibling	25.7	133/518	7.2	4.2 to 12.6
Grandparent	4.5	15/330	Reference	
Child	61.2	85/139	33.0	17.7 to 61.5
Partner	16.0	16/100	4.0	1.9 to 8.4
Other[a]	17.0	119/699	4.3	2.5 to 7.5
Vaccination status[b] (≤18 years)				
Fully vaccinated	23.8	138/581	0.11	0.07 to 0.17
Undervaccinated	52.9	64/121	0.38	0.22 to 0.68
Unvaccinated	74.5	76/102	Reference	
Received chemoprophylaxis[c]				
Yes	9.9	226/2,284	0.47	0.35 to 0.62
No	19.0	77/406	Reference	

CI: confidence interval.

[a] Caregiver, family friend or neighbour.

[b] Vaccination status was categorised as fully vaccinated (≥4 doses of vaccine), undervaccinated (<4 doses) or unvaccinated (no dose).

[c] Azithromycin was used.

period was 2 years, we expected to register 406 new cases during the study. Taking a mean of three household contacts (excluding the index case), we expected to register 1,218 household contacts. The median size of families in Spain is 2.5 members [25]; however, as other contacts in households, such as caregivers, were included, we decided to use a mean of three.

The rate of transmission in households, assuming an expected level of 10% [21], was estimated to a precision of ± 1.7%.

Data analysis

Primary cases and contacts were described using percentages with their 95% confidence intervals (CIs) for qualitative variables, and means and standard deviation (SD) for quantitative variables.

The rate of transmission with its 95% CI was calculated using the formula:

Formula 1

Secondary transmission rate = (Cases detected among the household contacts of primary cases/Total number of household contacts) × 100

Primary cases were not included in the numerator or the denominator.

The risk of transmission was studied according to the characteristics of primary cases and their household contacts using the chi-squared test for qualitative variables and the ANOVA or Kruskall tests for quantitative variables, with a level of significance of $p < 0.05$. The strength of an association was calculated using odds ratios (ORs) and their 95% CIs.

The vaccine effectiveness (only in household contacts aged 18 years or under) and chemoprophylaxis (in all household contacts) was studied using the formula: Effectiveness = (1 – OR) × 100. The estimated ORs were adjusted using an unconditional logistic regression model produced by eliminating variables using stepwise regression in which predictive variables were carried out by the automatic backward method starting with all candidate variables and eliminating variables from $p < 0.2$.

The variables evaluated in the models were vaccination status, use of chemoprophylaxis, age, sex and family relationship of the contacts, in addition to the sex, age and vaccination status of the primary case.

Ethical aspects

The study was approved by the Ethics Committee of the Hospital Sant Joan de Deu (code: PIC-79–11). All contacts and family members were informed about the study and gave their consent to participate.

Table 5

Multivariate analysis of the effectiveness of pertussis vaccination and chemoprophylaxis of household contacts in reducing household transmission, Catalonia and Navarre, Spain, 2012–13

Characteristic of household contact	Adjusted odds ratio[a]	95% CI	p value
Vaccination status[b] (≤18 years)			
Fully vaccinated	0.350	0.138 to 0.884	0.026
Undervaccinated	0.403	0.152 to 1.068	0.067
Unvaccinated	Reference		–
Received chemoprophylaxis[c]			
Yes	0.379	0.241 to 0.597	0.001
No	Reference		–

CI: confidence interval.

[a] Adjusted by age of contacts, sex of contacts, relationship with primary case, sex of primary case, age of primary case and pertussis vaccination status of primary case.

[b] Vaccination status was categorised as fully vaccinated (≥4 doses of vaccine), undervaccinated (<4 doses) or unvaccinated (no dose).

[c] Azithromycin was used.

Results

We studied 688 index cases, of whom 76.2% (524/688) were the primary cases in the household. The remainder (164/688) were secondary cases (household contacts). Thus the 688 primary cases studied (the first cases who became symptomatic in a household) comprised 524 index cases and 164 household contacts who were identified as primary cases once the study of the household was complete.

Of these 688 confirmed primary cases, 52.8% were female, 21.9% were aged under 1 year, 42.6% 1–10 years, 14.2% 11–18 years and 21.2% more than 18 years. Primary cases had the following symptoms: cough lasting more than 2 weeks (93.6%), paroxysmal cough (84.4%), post-tussive vomiting (40.1%), inspiratory stridor (37.6%), apnoea (21.9%) and fever (10.8%) (Table 1). The frequency of symptoms experienced by primary cases aged more than 18 years was slightly different: cough lasting more than 2 weeks (98.6%), paroxysmal cough (84.4%), post-tussive vomiting (23.1%), inspiratory stridor (30.6%), apnoea (20.4%) and fever (6.8%). Of the 688 primary cases, 15.3% were hospitalised, including 63.6% (96/151) of those aged under 1 year.

Laboratory confirmation (PCR and/or culture) was obtained for 73.3% (n = 504) of the primary cases and by epidemiological link in 26.7% (n = 184); 48.1% of cases were fully vaccinated (they had received ≥4 doses of vaccine), 13.1% were undervaccinated due to age, 2.2% were simply undervaccinated, 8.9% had received no vaccine dose and 9.6% were unvaccinated due to age (Table 1).

A total of 2,852 household contacts of the 688 primary cases were recorded, of whom 52.8% were female, 66.0% were older than 18 years, 7.3% were aged 11–18 years and 26.6% were under 11 years. About 71% of the contacts were cohabitants, i.e. they lived in the same household as the primary case. The most common family relationships among the contacts were being a mother (19.5%), father (17.9%) or sibling (18.2%) of the primary case. Some 64% of contacts aged ≤18 years were fully vaccinated, 13% were undervaccinated and 11% were unvaccinated (Table 2).

The household transmission rate (incidence of pertussis among household contacts) was 16.1% (459/2,852) and was slightly higher when the primary case was male (16.2%), but this difference was not statistically significant. Compared with data from primary cases aged under 1 year, the household transmission rate was higher when the primary case was aged 4–6 years (OR: 1.7; 95% CI: 1.1 to 2.6), 7–10 years (OR: 1.8; 95% CI: 1.3 to 2.6), 11–18 years (OR: 1.8; 95% CI: 1.2 to 2.6), 19–40 years (OR: 4.6; 95% CI: 3.2 to 6.6) and older than 40 years (OR = 4.3; 95% CI: 3.0 to 6.3). It was also higher when the primary case was undervaccinated, unvaccinated or of unknown vaccination status (OR: 1.3; 95% CI: 1.1 to 1.6), when compared with primary cases who were fully vaccinated (Table 3).

There was no statistically significant difference between the transmission rate in households with 2 or fewer contacts (19.5%), 3–4 contacts (16.0%) or more than 4 contacts (15.7%) ($p > 0.05$) (Table 3).

When looking at the transmission rate assessed according to variables of household contacts, the rate was slightly higher in male contacts (16.3%) than in female (15.9%), but this difference was not statistically significant. The rate was considerably higher in contacts aged under 1 year (OR: 24.6; 95% CI: 16.2 to 37.4), 1 year (OR: 8.8; 95% CI: 5.0 to 15.6), 2–3 years (OR: 3.6; 95% CI: 2.3 to 5.7), 4–6 years (OR: 3.0; 95% CI: 2.0 to 4.5), 7–10 years (OR: 2.6; 95% CI: 1.7 to 3.9) and 11–18 years (OR = 2.6; 95% CI: 1.7–3.9), compared with those aged more than 40 years (Table 4).

No difference in transmission rate was observed between contacts who were cohabitants and those who were non-cohabitants (with exposure for more than 2 hours in the household of the primary case). However, the transmission rate was higher in siblings (OR: 7.2; 95% CI: 4.2 to 12.6) and children (OR: 33.0; 95% CI: 17.7 to 61.5) of primary cases (Table 4).

Vaccine effectiveness in household contact aged ≤18 years was 89% (95% CI: 83 to 93) in reducing transmission in contacts vaccinated with 4 or fewer doses and 62% (95% CI: 32 to 78) in undervaccinated contacts.

Chemoprophylaxis in all contacts had an effectiveness of 53% (95% CI: 38 to 65) in avoiding new cases.

In the multivariate analysis, the effect of vaccination and chemoprophylaxis for contacts in avoiding new cases was still seen. Vaccine effectiveness in reducing transmission in contacts aged ≤ 18 years was 65.0% (95% CI: 11.6 to 86.2) for full vaccination and 59.7% (95% CI: −6.8 to 84.8%) for undervaccinated contacts. The adjusted effectiveness of chemoprophylaxis, based on adjusted ORs (Table 5), in all contacts was 62.1% (95% CI: 40.3 to 75.9).

Discussion

The results of this study show that the rate of household transmission of pertussis in Spain in 2012 and 2013 was high, especially in contacts aged under 18 years, siblings and children of a primary case, unvaccinated contacts and those who had not received chemoprophylaxis.

Household transmission of pertussis is known to be related to the characteristics of primary cases and their contacts [26]. We found increased transmission in households of primary cases aged 18–40 years and those older than 40 years. In the age group 18–40 years, this could be due to closer contact between children and the primary case, especially mothers, due to dependence [15,27]. For primary cases aged more than 40 years, the increased rate of transmission might be due to atypical clinical presentation, possibly resulting in important diagnostic delays and therefore more opportunities for transmission [16,27]. Lack of vaccination or undervaccination of the primary case also resulted in an increased transmission rate, as observed in other studies [28,29], showing that although full vaccination may not avoid the disease for some cases, it may reduce transmission from the primary case.

In our study, 35.4% of primary cases were adolescents (11–18 year-olds) or adults (> 18 years). Other studies also suggest that adolescents and adults are an important reservoir of the pathogen and source of transmission to children, who are more vulnerable to infection and susceptible to serious complications [28]. In a report published in 1995, Wirsing von König et al. studied pertussis cases in 122 homes in 1995 in an area of Germany with very low vaccination coverage and estimated that adults were the source of infection in 15% of cases [18]. Later, Baptista et al. studied pertussis cases in 57 homes in Recife, Brazil, in 2003 and found that adults were the primary source of infection in 21.1% of cases [21,30]. Deen et al. studied 39 homes and 255 exposed persons in Los Angeles, United States, in 1995: in 53% of households, the primary case was aged older than 12 years [31]. Sala-Farré et al. investigated 59 clusters in an area of Barcelona in 2011 and found that the most frequent primary cases were children aged 5–9 years (29%), followed by adults aged 30–39 years (22%) [32].

In Catalan children in 2001 hospitalised due to severe symptoms of pertussis [33], the source of infection was determined for 63% of cases and for 44.6% of those whose infection source was determined, the source was an adolescent or adult. It is recognised that adolescents and adults may act as a source of infection of children [14], but in these age groups the disease is often not diagnosed and is generally under-detected [34]. In a study in Massachusetts, United States, in 1981 to 1991 Marchant et al. [23,35] found an increase in the incidence of confirmed cases in adolescents aged 11–19 years from 3 per 100,000 population to 12.9 per 100,000 population, after facilitating general practitioners' access to serological diagnoses. In another study in Catalonia in 2013, the prevalence of *B. pertussis* infection in the previous 12 months was 1.8% in women of childbearing age (15–49 years), which suggests there is potentially a high risk for newborns [36]. Studies in various countries that included children hospitalised due to severe disease have shown that the most frequent sources of infection were mothers or other family members (fathers, teenage siblings and grandparents) who presented with coughing that had not been recognised as due to pertussis [16,21,27,37].

The rate of familial transmission from primary cases has been estimated in some studies. In the 1990s, Wirsing von König et al. found a high transmission rate of 26.7% in adult household contacts in an area of Germany with very low vaccination coverage [18] and in 2003, in Brazil, Baptista et al. found a rate of secondary transmission of 12.6% in adult household contacts [30].

In our study, we observed no differences between the number of contacts and transmission rate in the household. Similarly, in the study of Wirsing von König et al. the overall attack rate in adult contacts was independent of the family size [18] but an ecological study from 2009–13 in Minnesota, United States, reported a greater rate of pertussis in counties with a larger average household size [38].

The main characteristics of contacts with an increased transmission rate in our study were being 0–18 years of age, the sibling or child of a primary case, not vaccinated or undervaccinated and not receiving chemoprophylaxis. In terms of age, we observed a reduction of transmission in the 11–18-year age group and in adults compared with that of the other age groups. This may be due to vaccination with wP, as suggested by the World Health Organization (WHO) position paper on pertussis vaccines [9]. Reduced transmission in household adults was observed in the study of Baptista et al. in Recife, Brazil, in 2003. Some 87% of adults exposed to pertussis in the household did not acquire the disease: this was attributed to naturally acquired immunity [30]. In Catalonia and Navarre, five doses of aP were introduced into the official vaccination schedule in 2002 and therefore it may be assumed that most children aged under 11 years in our study were vaccinated with the aP. Specific responses to these changes, such as an adolescent booster dose (after the dose given at

age 4–6 years) and additional booster doses in adults, may be required.

Pertussis has re-emerged as an important public health concern in Europe since the current aP replaced the older wP. Warfel et al. showed that non-human primates receiving aP were protected from severe symptoms but not infection, and readily transmitted *B. pertussis* to contacts [39]. Key differences in T-cell memory suggest that aP vaccination induces a suboptimal immune response that is unable to prevent infection and provide a plausible explanation for pertussis resurgence [39]. Various studies suggest that attaining herd immunity will require the development of improved vaccination strategies that prevent *B. pertussis* colonisation and transmission [34,39,40].

The increased risk of transmission to siblings of primary cases seen in our study has also been observed by others [27,41]. The adjusted vaccine effectiveness of 65% in avoiding new cases in household contacts aged ≤ 18 years is similar to or higher than that observed in other studies [42]. Sheridan et al. found an effectiveness of 53% or 64%, depending on the method of calculation used [43]. However, a position paper by the World Health Organization (WHO) [9] and a systematic review published in the Cochrane database [44] suggest the effectiveness is somewhat higher: 84–85% in preventing typical whooping cough and 71–78% in preventing mild pertussis disease. The effectiveness of chemoprophylaxis with azithromycin in our study in preventing transmission was high (62.1%), suggesting that the detection of pertussis cases, analysing their contacts, and chemoprophylaxis may reduce household transmission, as has been suggested by others [21]. The evidence for the effectiveness of chemoprophylaxis in reducing transmission in household contacts is weak and based on expert opinion [45,46]. The results of our study and a recent cost–utility analysis [47] support the use of chemoprophylaxis in household contacts.

Our study has some limitations. First, it was based on notified cases of pertussis, which are known to be underdetected [35]. However, on the basis of the selection of study cases (confirmed cases with household contacts), an active search for contacts with pertussis symptoms was carried out using the survey and the taking of samples from all symptomatic household contacts of primary cases. To ensure all cases were detected, contacts were followed for 28 days from confirmation of the index case. Nevertheless, there may have been transmission due to asymptomatic cases beyond the 28 days of follow-up and the incidence of pertussis may be underestimated. We may not have identified individuals in a household who had recently been infected but may not have reported any specific symptoms. Thus, what is measured and presented in this study is the effectiveness of preventing clinically notifiable disease and not the prevention of infection. Second, vaccination status was collected by documented evidence of vaccination in an official document or medical records: some patients could have been classified as unvaccinated due to vaccination not being recorded, but if such a mistake applies equally to household contacts who remain healthy and those who become pertussis cases, it should not alter the estimated vaccine effectiveness. Third, chemoprophylaxis was recommended to all contacts without symptoms after detection of the index case. Some contacts who received chemoprophylaxis might appear as cases due to continuous exposure to other cases of pertussis in the household and, therefore, the effectiveness of chemoprophylaxis may be underestimated. However, our estimate was obtained after having followed routine pertussis control practices and may be a good estimate of the expected effectiveness when chemoprophylaxis is prescribed by public health services.

In conclusion, the results of our study suggest that in order to reduce household transmission household contacts should be investigated to detect secondary cases and administer chemoprophylaxis rapidly. All contacts who have not received the correct number of doses of pertussis vaccine according to the vaccination schedule should be vaccinated, in addition to receiving chemoprophylaxis. The incidence rate was lower in fully vaccinated individuals and therefore cases could be avoided in the future, although not the immediate future, as pertussis vaccine is not effective as post-exposure prophylaxis [47]. The previous pertussis vaccination status of cases and contacts is important in reducing the rate of household transmission. The administration of an additional dose of vaccine in adolescents and adults (especially those in contact with children) could also help to reduce the transmission rate [42]. Nevertheless, there is now increasing evidence that protection following booster doses of aP vaccines wanes faster in individuals primed with aP rather than with wP vaccines [9,34,39,40]. Such vaccination programmes have an impact in directly targeted populations, but there is as yet no substantial evidence that they have had an important impact on severe pertussis in infants. Thus, WHO recommends that national programmes consider vaccinating pregnant women with one dose of Tdap (in the second or third trimester and preferably at least 15 days before the end of the pregnancy) in addition to routine primary infant pertussis vaccination [9]. Ongoing surveillance of pertussis will be critical to monitor the changing epidemiology as the first 'all-aP'-primed cohorts reach adulthood.

Transmission of Pertussis in Households Working Group

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Conflict of interest

None declared.

Authors' contributions

PG, MG-C, GC, MA, JA, IB, JCast, PP, AD designed the study protocol and participated in the research call for funding for the study. PG, MG-C, GC, DT, PP, CM-A organised the logistics, sought approval from the bioethics committee and obtained informed consent from patients. PG, MG-C, DT, MA, JA, IB, NC, MC, M-RS-F, CR, MCarol, JF, GF, MJ, SM, RR, RT, IC, RS, SL, RB participated in the detection of index cases, recording and tracking of contacts, gathering epidemiological information and taking clinical samples and sending samples to the laboratory. CM-A, IJ, AV-R conducted and performed the microbiological analyses of clinical samples and sent the results to epidemiologists. PG, MG-C, DT, GC, JCay designed the databases of index cases and contacts and conducted the epidemiological and statistical analyses of the study. PG made a first draft of the paper and all authors made relevant contributions to successive versions. All authors reviewed and approved the final version of the article.

References

1. World Health Organization (WHO). WHO SAGE pertussis working group. Background paper. SAGE April 2014. Geneva: WHO; 2014. Available from: http://www.who.int/immunization/sage/meetings/2014/april/1_Pertussis_background_FINAL4_web.pdf?ua=
2. Liu L, Johnson HL, Cousens S, Perin J, Scott S, Lawn JE, et al. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet. 2012;379(9832):2151-61. DOI: 10.1016/S0140-6736(12)60560-1 PMID: 22579125
3. Cherry JD. Epidemic pertussis in 2012--the resurgence of a vaccine-preventable disease.N Engl J Med. 2012;367(9):785-7. DOI: 10.1056/NEJMp1209051 PMID: 22894554
4. Crespo I, Cardeñosa N, Godoy P, Carmona G, Sala MR, Barrabeig I, et al. Epidemiology of pertussis in a country with high vaccination coverage. Vaccine. 2011;29(25):4244-8. DOI: 10.1016/j.vaccine.2011.03.065 PMID: 21496465
5. Jenkinson D. Duration of effectiveness of pertussis vaccine: evidence from a 10 year community study.Br Med J (Clin Res Ed). 1988;296(6622):612-4. DOI: 10.1136/bmj.296.6622.612 PMID: 3126927
6. Witt MA, Katz PH, Witt DJ. Unexpectedly limited durability of immunity following acellular pertussis vaccination in preadolescents in a North American outbreak.Clin Infect Dis. 2012;54(12):1730-5. DOI: 10.1093/cid/cis287 PMID: 22423127
7. Cortese MM, Baughman AL, Zhang R, Srivastava PU, Wallace GS. Pertussis hospitalizations among infants in the United States, 1993 to 2004.Pediatrics. 2008;121(3):484-92. DOI: 10.1542/peds.2007-1393 PMID: 18310196
8. Lambert HJ. Epidemiology of a small pertussis outbreak in Kent County, Michigan.Public Health Rep. 1965;80(4):365-9. DOI: 10.2307/4592424 PMID: 14279983
9. Pertussis vaccines: WHO position paper - September 2015. Wkly Epidemiol Rec. 2015;90(35):433-58.PMID: 26320265
10. Gustafsson L, Hessel L, Storsaeter J, Olin P. Long-term follow-up of Swedish children vaccinated with acellular pertussis vaccines at 3, 5, and 12 months of age indicates the need for a booster dose at 5 to 7 years of age.Pediatrics. 2006;118(3):978-84. DOI: 10.1542/peds.2005-2746 PMID: 16950988
11. Klein NP, Bartlett J, Fireman B, Rowhani-Rahbar A, Baxter R. Comparative effectiveness of acellular versus whole-cell pertussis vaccines in teenagers.Pediatrics. 2013;131(6):e1716-22. DOI: 10.1542/peds.2012-3836 PMID: 23690518
12. Klein NP, Bartlett J, Rowhani-Rahbar A, Fireman B, Baxter R. Waning protection after fifth dose of acellular pertussis vaccine in children.N Engl J Med. 2012;367(11):1012-9. DOI: 10.1056/NEJMoa1200850 PMID: 22970945
13. Sizaire V, Garrido-Estepa M, Masa-Calles J, Martinez de Aragon MV. Increase of pertussis incidence in 2010 to 2012 after 12 years of low circulation in Spain.Euro Surveill. 2014;19(32):20875. DOI: 10.2807/1560-7917.ES2014.19.32.20875 PMID: 25139074
14. Bisgard KM, Pascual FB, Ehresmann KR, Miller CA, Cianfrini C, Jennings CE, et al. Infant pertussis: who was the source? Pediatr Infect Dis J. 2004;23(11):985-9. DOI: 10.1097/01.inf.0000145263.37198.2b PMID: 15545851
15. Wendelboe AM, Njamkepo E, Bourillon A, Floret DD, Gaudelus J, Gerber M, et al. , Infant Pertussis Study Group. Transmission of Bordetella pertussis to young infants.Pediatr Infect Dis J. 2007;26(4):293-9. DOI: 10.1097/01.inf.0000258699.64164.6d PMID: 17414390
16. Kowalzik F, Barbosa AP, Fernandes VR, Carvalho PR, Avila-Aguero ML, Goh DYT, et al. Prospective multinational study of pertussis infection in hospitalized infants and their household contacts. Pediatr Infect Dis J. 2007;26(3):238-42. DOI: 10.1097/01.inf.0000256750.07118.ee PMID: 17484221
17. Deen JL, Mink CA, Cherry JD, Christenson PD, Pineda EF, Lewis K, et al. Household contact study of Bordetella pertussis infections. Clin Infect Dis. 1995;21(5):1211-9. DOI: 10.1093/clinids/21.5.1211 PMID: 8589145
18. Wirsing von König CH, Postels-Multani S, Bock HL, Schmitt HJ. Pertussis in adults: frequency of transmission after household exposure.Lancet. 1995;346(8986):1326-9. DOI: 10.1016/S0140-6736(95)92343-8 PMID: 7475771
19. Tiwari T, Murphy TV, Moran J, National Immunization Program, CDC. Recommended antimicrobial agents for the treatment and postexposure prophylaxis of pertussis: 2005 CDC Guidelines. MMWR Recomm Rep. 2005;54(RR-14):1-16.PMID: 16340941
20. Instituto Nacional de Estadística (INE). Cifras de población y censos demográficos. [National Statistics Institute. Population figures and Demographic Censuses and Population figures]. 2016 data. Madrid: INE. [Accessed 31 Oct 2016]. Spanish. Available from: http://www.ine.es/inebaseDYN/cp30321/cp_inicio.htm
21. Baptista PN, Magalhães VS, Rodrigues LC. Children with pertussis inform the investigation of other pertussis cases among contacts.BMC Pediatr. 2007;7(1):21. DOI: 10.1186/1471-2431-7-21 PMID: 17518997
22. European Centre for Disease Prevention and Control (ECDC). Systematic review on the incubation and infectiousness/shedding period of communicable diseases in children. Stockholm: ECDC; 2016. Available from: http://ecdc.europa.eu/en/publications/publications/systematic-review-incubation-period-shedding-children.pdf
23. Roorda L, Buitenwerf J, Ossewaarde JM, van der Zee A. A real-time PCR assay with improved specificity for detection and discrimination of all clinically relevant Bordetella species by the presence and distribution of three Insertion Sequence elements.BMC Res Notes. 2011;4(1):11. DOI: 10.1186/1756-0500-4-11 PMID: 21255383
24. Resumen anual 2011. [Annual summary 2011]. Boletin Epidemiológico Semanal. 2011;19(1):255. Spanish. Available from: http://revista.isciii.es/index.php/bes/article/view/689/721
25. Las formas de la convivencia. [Ways of cohabitation. National Statistics Institute.]. Boletin informative del Instituto Nacional de Estadistica. July 2014. [Accessed 31 Oct 2016]. Available

from: http://www.ine.es/ss/Satellite?L=es_ES&c=INECifrasINE_C&cid=1259944407896&p=1254735116567&pagename=ProductosYServicios%2FINECifrasINE_C%2FPYSDetalleCifrasINE

26. Wirsing von König CH, Postels-Multani S, Bogaerts H, Bock HL, Laukamp S, Kiederle S, et al. Factors influencing the spread of pertussis in households. Eur J Pediatr. 1998;157(5):391-4. DOI: 10.1007/s004310050836 PMID: 9625336
27. de Greeff SC, de Melker HE, Westerhof A, Schellekens JF, Mooi FR van BM, van Boven M. Estimation of household transmission rates of pertussis and the effect of cocooning vaccination strategies on infant pertussis.Epidemiology. 2012;23(6):852-60. DOI: 10.1097/EDE.0b013e31826c2b9e PMID: 23018969
28. Ward JI, Cherry JD, Chang SJ, Partridge S, Lee H, Treanor J, et al. Efficacy of an acellular pertussis vaccine among adolescents and adults. N Engl J Med. 2005;353(15):1555-63. DOI: 10.1056/NEJMoa050824 PMID: 16221778
29. Baptista PN, Magalhães V, Rodrigues LC, Rocha MW, Pimentel AM. Pertussis vaccine effectiveness in reducing clinical disease, transmissibility and proportion of cases with a positive culture after household exposure in Brazil. Pediatr Infect Dis J. 2006;25(9):844-6. DOI: 10.1097/01.inf.0000232642.25495.95 PMID: 16940847
30. Baptista PN, Magalhães VS, Rodrigues LC. The role of adults in household outbreaks of pertussis.Int J Infect Dis. 2010;14(2):e111-4. DOI: 10.1016/j.ijid.2009.03.026 PMID: 19559636
31. Deen JL, Mink CA, Cherry JD, Christenson PD, Pineda EF, Lewis K, et al. Household contact study of Bordetella pertussis infections. Clin Infect Dis. 1995;21(5):1211-9. DOI: 10.1093/clinids/21.5.1211 PMID: 8589145
32. Sala-Farré MR, Arias-Varela C, Recasens-Recasens A, Simó-Sanahuja M, Muñoz-Almagro C, Pérez-Jové J. Pertussis epidemic despite high levels of vaccination coverage with acellular pertussis vaccine.Enferm Infecc Microbiol Clin. 2015;33(1):27-31. DOI: 10.1016/j.eimc.2013.09.013 PMID: 24216286
33. Moraga F, Roca J, Méndez C, Rodrigo C, Pineda V, Martinez A, et al. , TOSCA Study Group. Epidemiology and surveillance of pertussis among infants in Catalonia, Spain, during 1997-2001.Pediatr Infect Dis J. 2005;24(6):510-3. DOI: 10.1097/01.inf.0000164701.50766.62 PMID: 15933560
34. Althouse BM, Scarpino SV. Asymptomatic transmission and the resurgence of Bordetella pertussis.BMC Med. 2015;13(1):146. DOI: 10.1186/s12916-015-0382-8 PMID: 26103968
35. Marchant CD, Loughlin AM, Lett SM, Todd CW, Wetterlow LH, Bicchieri R, et al. Pertussis in Massachusetts, 1981-1991: incidence, serologic diagnosis, and vaccine effectiveness. J Infect Dis. 1994;169(6):1297-305. DOI: 10.1093/infdis/169.6.1297 PMID: 8195607
36. Plans P, Álvarez E, de Ory F, Campins M, Payà T, Balfagón P, et al. Prevalence of antibody to Bordetella pertussis in neonates and prevalence of recent pertussis infection in pregnant women in Catalonia (Spain) in 2003 and 2013. Pediatr Infect Dis J. 2014;33(11):1114-8. DOI: 10.1097/INF.0000000000000413 PMID: 24871642
37. McGuiness CB, Hill J, Fonseca E, Hess G, Hitchcock W, Krishnarajah G. The disease burden of pertussis in adults 50 years old and older in the United States: a retrospective study. BMC Infect Dis. 2013;13(1):32. DOI: 10.1186/1471-2334-13-32 PMID: 23343438
38. Iroh Tam PY, Menk JS, Hughes J, Kulasingam SL. An ecological analysis of pertussis disease in Minnesota, 2009-2013. Epidemiol Infect. 2016;144(4):847-55. DOI: 10.1017/S0950268815002046 PMID: 26330135
39. Warfel JM, Zimmerman LI, Merkel TJ. Acellular pertussis vaccines protect against disease but fail to prevent infection and transmission in a nonhuman primate model.Proc Natl Acad Sci USA. 2014;111(2):787-92. DOI: 10.1073/pnas.1314688110 PMID: 24277828
40. van den Biggelaar AH, Poolman JT. Predicting future trends in the burden of pertussis in the 21st century: implications for infant pertussis and the success of maternal immunization.Expert Rev Vaccines. 2016;15(1):69-80. DOI: 10.1586/14760584.2016.1105136 PMID: 26559122
41. Bertilone C, Wallace T, Selvey LA. Finding the 'who' in whooping cough: vaccinated siblings are important pertussis sources in infants 6 months of age and under.Commun Dis Intell Q Rep. 2014;38(3):E195-200.PMID: 25391405
42. Baxter R, Bartlett J, Rowhani-Rahbar A, Fireman B, Klein NP. Effectiveness of pertussis vaccines for adolescents and adults: case-control study.BMJ. 2013;347(jul17 1):f4249. DOI: 10.1136/bmj.f4249 PMID: 23873919
43. Sheridan SL, Ware RS, Grimwood K, Lambert SB. Unexpectedly limited durability of immunity following acellular pertussis vaccination in preadolescents in a North American outbreak. Clin Infect Dis. 2012;55(10):1434-5, author reply 1435-6. DOI: 10.1093/cid/cis672 PMID: 22871826
44. Zhang L, Prietsch SO, Axelsson I, Halperin SA, et al. Acellular vaccines for preventing whooping cough in children. Cochrane Database Syst Rev. 2014; (9):CD001478.PMID: 25228233
45. Dodhia H, Crowcroft NS, Bramley JC, Miller E. UK guidelines for use of erythromycin chemoprophylaxis in persons exposed to pertussis.J Public Health Med. 2002;24(3):200-6. DOI: 10.1093/pubmed/24.3.200 PMID: 12831090
46. Friedman DS, Curtis CR, Schauer SL, Salvi S, Klapholz H, Treadwell T, et al. Surveillance for transmission and antibiotic adverse events among neonates and adults exposed to a healthcare worker with pertussis. Infect Control Hosp Epidemiol. 2004;25(11):967-73. DOI: 10.1086/502328 PMID: 15566032
47. Thampi N, Gurol-Urganci I, Crowcroft NS, Sander B. Pertussis post-exposure prophylaxis among household contacts: a cost-utility analysis.PLoS One. 2015;10(3):e0119271. DOI: 10.1371/journal.pone.0119271 PMID: 25747269

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Letter to the editor: Potential causes of the decreased effectiveness of the influenza A(H1N1)pdm09 strain in live attenuated influenza vaccines

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To the editor: We greatly appreciate the editorial by Penttinen and Friede summarising the data regarding recent observations in the United States (US) of decreased effectiveness of the influenza A(H1N1)pdm09 strains (A/California/7/2009 and A/Bolivia/559/2013) included in live attenuated influenza vaccines (LAIV) [1]. Multiple hypotheses have been suggested as potential explanations for the reduced effectiveness compared with inactivated influenza vaccines (IIV). The most frequently cited hypotheses include poor replicative fitness of the A(H1N1)pdm09 LAIV strains, vaccine–virus interference in the quadrivalent formulation, reduced LAIV replication due to preexisting anti-influenza immunity from prior influenza vaccinations, and poor thermostability of A(H1N1)pdm09 LAIV strains. We have systematically evaluated each of these hypotheses and would like to share our assessments in case they might benefit ongoing international scientific discussions regarding LAIV effectiveness.

Based on evidence presently available to us, we believe that reduced replicative fitness of the A/California and A/Bolivia (H1N1)pdm09 LAIV strains is the most probable root cause for the reduced vaccine effectiveness (VE). From 2010/11 through 2013/14, LAIV VE in children aged 2–17 years against matched A(H3N2) and B strains has been comparable to that observed with IIV [2,3]. In 2014/15, LAIV4 VE against mismatched A(H3N2) strains was low, similar to that observed with IIV [2], and similar to that of LAIV3 against mismatched A(H3N2) strains that are ≥ 8-fold different by haemagglutination-inhibition assay [4,5]. Laboratory studies that we have conducted since April 2016 show that A/California and A/Bolivia strains have reduced replication in a human alveolar cell line and in primary human nasal epithelium air-liquid cultures, as well as reduced binding to α2,6-linked sialic acid receptors—the primary receptor for influenza viruses in the human upper respiratory tract. Consequently, we are actively working to identify a new A(H1N1)pdm09 LAIV strain with replicative fitness superior to that of A/California and A/Bolivia and similar to the replicative fitness of LAIV strains that previously demonstrated high levels of effectiveness in children.

In the context of reduced replicative fitness, vaccine–virus interference may have contributed to the observed reduced VE. However, vaccine–virus interference specific to the quadrivalent formulation appears to be an unlikely root cause of the reduced VE with LAIV. LAIV3 demonstrated reduced VE against A(H1N1)pdm09 in 2010/11 in the US [2] and 2012/13 in Germany [6]. Additionally, no VE was observed against A(H1N1)pdm09 strains in a randomised placebo-controlled study in children aged 2–5 years with trivalent A/Leningrad LAIV [7]. As reduced VE against A(H1N1)pdm09 strains was observed with trivalent LAIV formulations, any effects of vaccine–virus interference do not appear specific to the quadrivalent LAIV formulation.

Because rates of vaccine coverage in the US have historically been higher compared with European countries, questions have been raised regarding the role of prior vaccination in the reduced A(H1N1)pdm09 effectiveness [1]. Available data suggest that preexisting anti-influenza immunity due to prior vaccination is an unlikely root cause of the reduced VE observed with LAIV. In 2013/14 and 2015/16, the effect of prior season influenza vaccination on LAIV VE was evaluated in the US-based Centers for Disease Control and Prevention Flu VE and Influenza Clinical Investigation for Children (ICICLE) studies [8-10]. No statistically significant effect of prior season vaccination on LAIV VE was observed in either study in any season. Additionally, in the ICICLE study and in a large cohort study of children aged 24–35 months in Finland, most LAIV recipients were previously vaccinated. VE estimates trended higher among children vaccinated against influenza compared with unvaccinated children in the prior season in the ICICLE 2013/14 study (19% (95% confidence interval

(CI): -80 to 64) vs 9% (95%CI: -161 to 68)); the ICICLE 2015/16 study (60% (95% CI: 1 to 84) vs 35% (95% CI: -206 to 86)), and the Finland study (74% (95% CI: 48 to 87) vs 25% (95% CI: -27 to 56)) [11].

In 2013/14, with LAIV4 containing the A/California strain, a statistically significant correlation was observed between reduced LAIV VE against (H1N1) pdm09 viruses and higher outdoor temperatures during LAIV lot unloading at US distributors [12]. In laboratory experiments, A/California demonstrated increased heat degradation [13], including experiments that simulated heat exposures that may have occurred during US distribution (33 °C for 4 hours). Environmental heat exposure has also been suggested as a contributing factor to the lack of LAIV VE against A(H1N1)pdm09 viruses in a randomised placebo-controlled study in children aged <5 years with trivalent A/Leningrad LAIV [14]. However, reduced VE was also observed in 2015/16 with A/Bolivia, the strain chosen to replace A/California based on its being more heat stable [1]. Consequently, although the reduced thermostability of A/California appears to have contributed to the low VE observed in 2013/14 in the US, it cannot explain the observations of reduced VE against A(H1N1)pdm09 strains in 2015/16 as A/Bolivia was thermostable.

We have initiated a multifaceted scientific investigation into the causes of the recently observed reduced effectiveness of LAIV, with the goal of identifying a more effective A(H1N1)pdm09 LAIV strain for potential inclusion in the 2017/18 LAIV formulation. All potential hypotheses continue to be evaluated. We welcome the input and support of the multiple stakeholders involved, including national public health agencies, the World Health Organization, and additional external scientific experts, as we work together to ensure that VE of LAIV is improved in future influenza seasons

Conflict of interest

Christopher S. Ambrose, Helen Bright, and Raburn Mallory are full-time employees of MedImmune.

Authors' contributions

All authors participated in the drafting of this letter, reviewed it critically for important intellectual content, and approved the final version.

References

1. Penttinen PM, Friede MH. Decreased effectiveness of the influenza A(H1N1)pdm09 strain in live attenuated influenza vaccines: an observational bias or a technical challenge?Euro Surveill. 2016;21(38):30350. DOI: 10.2807/1560-7917.ES.2016.21.38.30350 PMID: 27684999
2. Chung JR, Flannery B, Thompson MG, Gaglani M, Jackson ML, Monto AS, et al. Seasonal effectiveness of live attenuated and inactivated influenza vaccine. Pediatrics. 2016;137(2):e20153279. DOI: 10.1542/peds.2015-3279 PMID: 26738884
3. Caspard H, Gaglani M, Clipper L, Belongia EA, McLean HQ, Griffin MR, et al. Effectiveness of live attenuated influenza vaccine and inactivated influenza vaccine in children 2-17 years of age in 2013-2014 in the United States. Vaccine. 2016;34(1):77-82. DOI: 10.1016/j.vaccine.2015.11.010 PMID: 26589519
4. Lum LC, Borja-Tabora CF, Breiman RF, Vesikari T, Sablan BP, Chay OM, et al. Influenza vaccine concurrently administered with a combination measles, mumps, and rubella vaccine to young children. Vaccine. 2010;28(6):1566-74. DOI: 10.1016/j.vaccine.2009.11.054 PMID: 20003918
5. Klick B, Durrani S, Chan KH, Ip DK, Chou ES, Kwok HK, et al. Live attenuated seasonal and pandemic influenza vaccine in school-age children: a randomized controlled trial. Vaccine. 2013;31(15):1937-43. DOI: 10.1016/j.vaccine.2013.02.017 PMID: 23434387
6. Helmeke C, Gräfe L, Irmscher HM, Gottschalk C, Karagiannis I, Oppermann H. Effectiveness of the 2012/13 trivalent live and inactivated influenza vaccines in children and adolescents in Saxony-Anhalt, Germany: a test-negative case-control study.PLoS One. 2015;10(4):e0122910. DOI: 10.1371/journal.pone.0122910 PMID: 25885063
7. Victor JC, Lewis KD, Diallo A, Niang MN, Diarra B, Dia N, et al. Efficacy of a Russian-backbone live attenuated influenza vaccine among children in Senegal: a randomised, double-blind, placebo-controlled trial. Lancet Glob Health. 2016;S2214-109X(16)30201-7.PMID: 27746224
8. Centers for Disease Control and Prevention (CDC). Advisory Committee on Immunization Practices (ACIP) Summary Report. Meeting held: October 29–30, 2014; Atlanta, GA: CDC; 2014. Available from: http://www.cdc.gov/vaccines/acip/meetings/downloads/min-archive/min-2014-10.pdf
9. Flannery B. LAIV vs IIV effectiveness: Summary of evidence since 2009. Presented at: Centers for Disease Control and Prevention Advisory Committee on Immunization Practices (ACIP) Meeting; 2016 Jun 22–23; Atlanta, GA. Available from: http://www.cdc.gov/vaccines/acip/meetings/downloads/slides-2016-06/influenza-07-flannery.pdf
10. Caspard H, Belongia EA, Bernatoniene J, Clipper L, Congeni B, Faust S, et al. Multicenter study of the effectiveness of live attenuated influenza vaccine and inactivated influenza vaccine in children in 2015–2016 in the United States. Poster presented at: Options for Influenza Meeting; 2016 Aug 24–28; Chicago, IL.
11. Nohynek H, Baum U, Syrjänen R, Ikonen N, Sundman J, Jokinen J. Effectiveness of the live attenuated and the inactivated influenza vaccine in two-year-olds - a nationwide cohort study Finland, influenza season 2015/16.Euro Surveill. 2016;21(38):30346. DOI: 10.2807/1560-7917.ES.2016.21.38.30346 PMID: 27684447
12. Caspard H, Coelingh KL, Mallory RM, Ambrose CS. Association of vaccine handling conditions with effectiveness of live attenuated influenza vaccine against H1N1pdm09 viruses in the United States.Vaccine. 2016;34(42):5066-72. DOI: 10.1016/j.vaccine.2016.08.079 PMID: 27613072
13. Cotter CR, Jin H, Chen Z. A single amino acid in the stalk region of the H1N1pdm influenza virus HA protein affects viral fusion, stability and infectivity.PLoS Pathog. 2014;10(1):e1003831. DOI: 10.1371/journal.ppat.1003831 PMID: 24391498
14. Isakova-Sivak I. Use of live attenuated influenza vaccines in young children in resource-poor settings.Lancet Glob Health. 2016;S2214-109X(16)30247-9.PMID: 27746227