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Persistent detection of dengue virus RNA in vaginal secretion of a woman returning from Sri Lanka to Italy, April 2017

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We describe the dynamics of dengue virus (DENV) infection in a woman in her mid-30s who developed fever after returning from Sri Lanka to Italy in April 2017. Laboratory testing demonstrated detectable DENV-RNA in plasma, urine, saliva, vaginal secretion. Persistent shedding of DENV-RNA was demonstrated in vaginal secretion, and DENV-RNA was detectable in the pelleted fraction up to 18 days from symptom onset. These findings give new insights into DENV vaginal shedding and vertical transmission.

We present a case of primary dengue fever (DF) in a Caucasian woman returning from Sri Lanka to Italy in April 2017. Dengue virus (DENV) RNA was persistently detected in vaginal secretion up to 18 days from symptom onset (FSO).

Case report

In April 2017, a Caucasian woman in her mid-30s returning to Italy from a 19-day travel in Sri Lanka, experienced a 3-day course of fever (›38.5 °C), arthralgia, weakness and headache. On admission at the National Institute for Infectious Diseases Lazzaro Spallanzani in Rome, Italy (day 3 FSO), a commercial dengue rapid test (Dengue DUO, Standard Diagnostics Inc., Kyonggi-do, Korea), detecting specific IgG, IgM and non-structural (NS)-1 protein, revealed NS-1 antigen reactivity only. Routine laboratory tests showed transient leukopenia and thrombocytopenia, with slight increase of liver enzymes and alterations of coagulation parameters (Figure).

On day 4 FSO, DENV-specific IgG and IgM, assessed by indirect immune fluorescence assay (IFA, Arboviral Fever Mosaic-2, IgM and IgG, Euroimmun, Hamburg, Germany), were below the detection threshold (1/20), and real-time PCR for DENV (CDC DENV-1–4 Real-Time RT-PCR Assay, Atlanta, United States (US)) was positive in serum (cycle threshold (Ct): 22); hence the diagnosis was primary DENV infection. Viral RNA was also detected in urine (Ct: 34.61), saliva (Ct: 33.55) and vaginal secretion (Ct: 30.71). The latter was collected by flocked swab and immediately suspended in 2 mL of vaginal swab transport medium (VSTM) at 4 °C in a 15-mL sterile tube. Moreover, a pan-flavivirus genus-specific nested RT-PCR targeting the NS-5 gene (modified from [1]) followed by the amplicon sequencing, showed DENV type 2 in all the collected samples (saliva, urine, serum and vaginal swab). The virological investigation for DENV was repeated on longitudinally collected samples (Table).

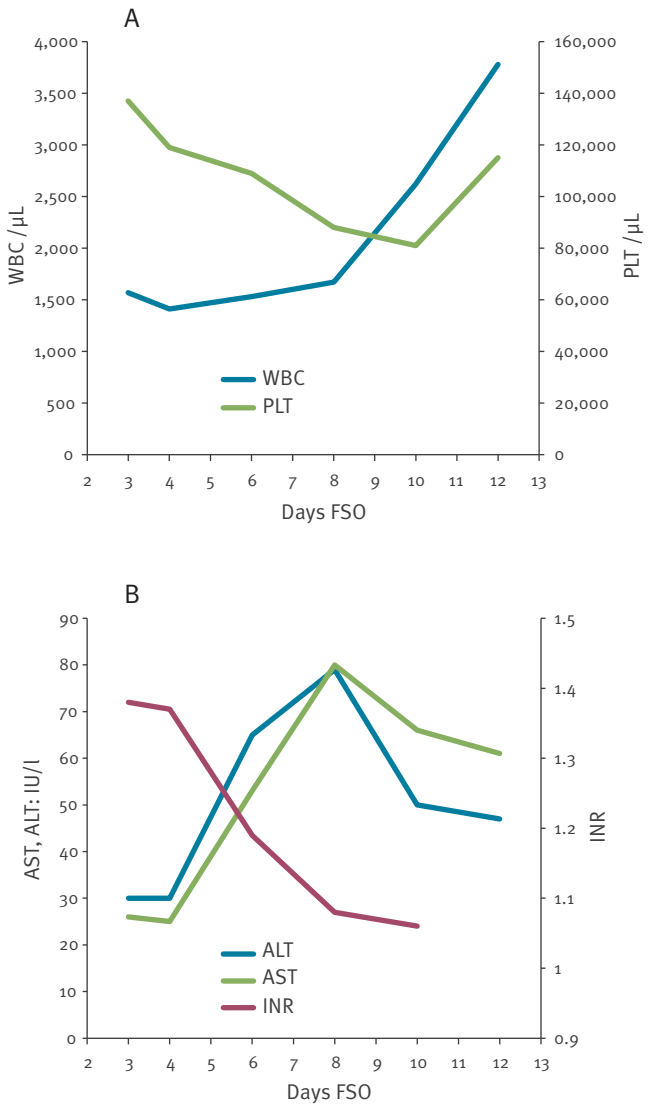
Rapid test, thin and thick smear and PCR for malaria were all negative; chikungunya and Zika virus serologies were negative as well as specific PCR in serum and urine. Epstein-Barr virus and Cytomegalovirus serology indicated past infections, with negative IgM for both viruses.

On day 10 FSO, serum, urine, saliva, and vaginal swab were collected, and DENV-RNA was still detectable in all the samples (serum Ct: 32.96; urine Ct: 31.50; saliva Ct: 37.29; VSTM Ct: 35.47). VSTM was centrifuged at 1,500 rpm for 10 minutes to obtain supernatant fractions (SNF) and pelleted fractions (PeF). Total RNA was extracted from SNF and PeF using the COBAS AmpliPrep Total Nucleic Acid Isolation Kit (Roche, Indianapolis, Indiana, US) and Trizol (Life Technologies, Stockholm, Sweden) respectively, according to the manufacturer's instructions. RT-PCR for DENV-RNA resulted positive in SNF and PeF with a Ct of 35.47 and 33.34, respectively.

On day 18 FSO, DENV-RNA was detectable only in urine (Ct: 32.53), while it was undetectable in serum, saliva and VSTM. After separation of VSTM, DENV-RNA was

Figure

Laboratory tests performed during hospitalisation, case of dengue fever, Italy, April 2017



FSO: from symptom onset.

A: white blood cell (WBC) and platelet (PLT) absolute counts.

B: liver enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), international normalised ratio (INR).

undetectable in SNF, while it was detected in PeF (Ct: 35.89).

On day 36 FSO, DENV-RNA was no longer detectable in serum, urine, saliva, total VSTM and SNF and PeF (Table).

Specific IgM became detectable on day 10 FSO, and IgG and IgM titres rose on day 18 FSO (1/160 and 1/80, respectively) and on day 36 (1/320 and 1/160, respectively). Viral isolation from vaginal samples using VeroE6 cell culture was not successful.

Background

DF is an arthropod-borne viral infection, transmitted to humans through mosquito bites and caused by DENV, a single stranded, positive-sense RNA virus, belonging to the genus Flavivirus, like Zika virus (ZIKV) and West Nile virus (WNV). Four serotypes (from 1 to 4) have been identified so far and each DENV serotype accounts for multiple phylogenetically-related genotypes [2]. DENV is widely diffused, causing more than 58 million symptomatic infections in 2013 [3]. Infection during pregnancy can cause severe maternal and neonatal complications [4,5], However, no fetal abnormalities have been reported so far after DENV infection in pregnant women. Vertical transmission has been described in several reports [6,7], whereas sexual transmission of DENV has never been reported.

Discussion

The recent ZIKV outbreak evidenced the occurrence of vertical transmission of the virus with fetal abnormalities [8] and male-to-woman, woman-to-male and male-to-male sexual transmission [9], and highlighted the presence of infectious virus in almost all body secretions, including those from male and female genital tract [10-13].

Here we report on DENV-RNA detection in vaginal secretion of an acute case of primary DF. Moreover, we report the detection of viral RNA with the pellet fraction of vaginal secretion after centrifugation. This could reflect DENV association to vaginal epithelial cells, although association with other components of the pellet may not be ruled out. Although vaginal shedding of DENV was protracted up to 18 days FSO, we were not able to isolate replication competent virus from the different fractions derived from genital secretion, probably because of low viral loads detected in the vaginal swab, influence of sample pH and virus particles degradation. However, our findings could give a deeper insight in DENV sexual and vertical transmission.

Little is known about vertical transmission of DENV and a recent meta-analysis by Xiong et al. [14], failed to demonstrate that maternal DENV infection during pregnancy might increase the risk of premature birth, low birth weight, miscarriage and stillbirth. DENV has been identified in newborns after Caesarean section and the virus was isolated from umbilical cord blood, indicating the possibility of intrauterine acquisition of the infection; the presence of the virus in the vaginal mucosa, shown in our patient, is consistent with the possibility that DENV can be vertically transmitted also during vaginal delivery, similarly to genital herpes [15].

Interestingly, an in vitro study by Chan et al. [16] demonstrated the ability of DENV type 2 to replicate in human cell lines derived from the cervix (HeLa) while no viral growth was observed in experimental infection of placenta (JEG-3), endometrium (HOSE6–3), prostate (LNCaP) and testis (833KE) cell lines. Conversely,

Table

Dengue virus-RNA detection and serology at different time points, case of dengue fever, Italy, April 2017

Days FSO	DENV-RNA RT-PCR						DENV serology		
	Serum	Urine	Saliva	Vaginal swab			IgM	IgG	NS1
				Total VSTM	PeF	SNF			
3	NA	NA	NA	NA	NA	NA	Neg[a]	Neg[a]	Pos[a]
4	Pos (Ct: 22.00)	Pos (Ct: 34.61)	Pos (Ct: 33.55)	Pos (Ct: 30.71)	NA	NA	<1:20	<1:20	NA
10	Pos (Ct: 32.96)	Pos (Ct: 31.50)	Pos (Ct: 37.29)	Pos (Ct: 35.47)	Pos (Ct: 35.47)	Pos (Ct: 33.34)	1:20	<1:20	NA
18	Neg	Pos (Ct: 32.53)	Neg	Neg	Pos (Ct: 35.89)	Neg	1:80	1:160	NA
36	Neg	Neg	Neg	Neg	Neg	Neg	1:160	1:320	NA

[a] Rapid test.

Ct: cycle threshold; DENV: dengue virus; FSO: from symptom onset; NA: not available; Neg: negative; PeF: pelleted fraction; Pos: positive; SNF: supernatant fraction; VSTM: vaginal swab transport medium.

all the above-mentioned cell lines were susceptible to ZIKV [16].

Further investigations in individuals with acute DF are needed in order to understand the implications of DENV genital shedding on vertical and sexual transmission. Viral isolation and innovative molecular methods to detect DENV in the replicative phase represent essential steps in this process.

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Conflict of interest

None declared.

Authors' contributions

Marco Iannetta, Maria Musso, Alessandra D'Abramo, Laura Scorzolini and Pierangelo Chinello were the physicians in charge of the patient; Eleonora Lalle, Fabrizio Carletti and Concetta Castilletti were the virologists in charge of the virological assays for Dengue virus diagnosis; Marco Iannetta wrote the manuscript. Maria R. Capobianchi, who is responsible for the Laboratory of Virology, Giuseppe Ippolito, who supervises all the clinical and translational research on emerging and re-emerging pathogens and Emanuele Nicastri, who is Head of the High Intensity of Care and Highly Contagious Infectious Disease Unit, contributed to the discussion and reviewed the manuscript.

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Large measles outbreak introduced by asylum seekers and spread among the insufficiently vaccinated resident population, Berlin, October 2014 to August 2015

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The largest measles outbreak in Berlin since 2001 occurred from October 2014 to August 2015. Overall, 1,344 cases were ascertained, 86% (with available information) unvaccinated, including 146 (12%) asylum seekers. Median age was 17 years (interquartile range: 4–29 years), 26% were hospitalised and a 1-year-old child died. Measles virus genotyping uniformly revealed the variant 'D8-Rostov-Don' and descendants. The virus was likely introduced by and initially spread among asylum seekers before affecting Berlin's resident population. Among Berlin residents, the highest incidence was in children aged < 2 years, yet most cases (52%) were adults. Post-exposure vaccinations in homes for asylum seekers, not always conducted, occurred later (median: 7.5 days) than the recommended 72 hours after onset of the first case and reached only half of potential contacts. Asylum seekers should not only have non-discriminatory, equitable access to vaccination, they also need to be offered measles vaccination in a timely fashion, i.e. immediately upon arrival in the receiving country. Supplementary immunisation activities targeting the resident population, particularly adults, are urgently needed in Berlin.

Introduction

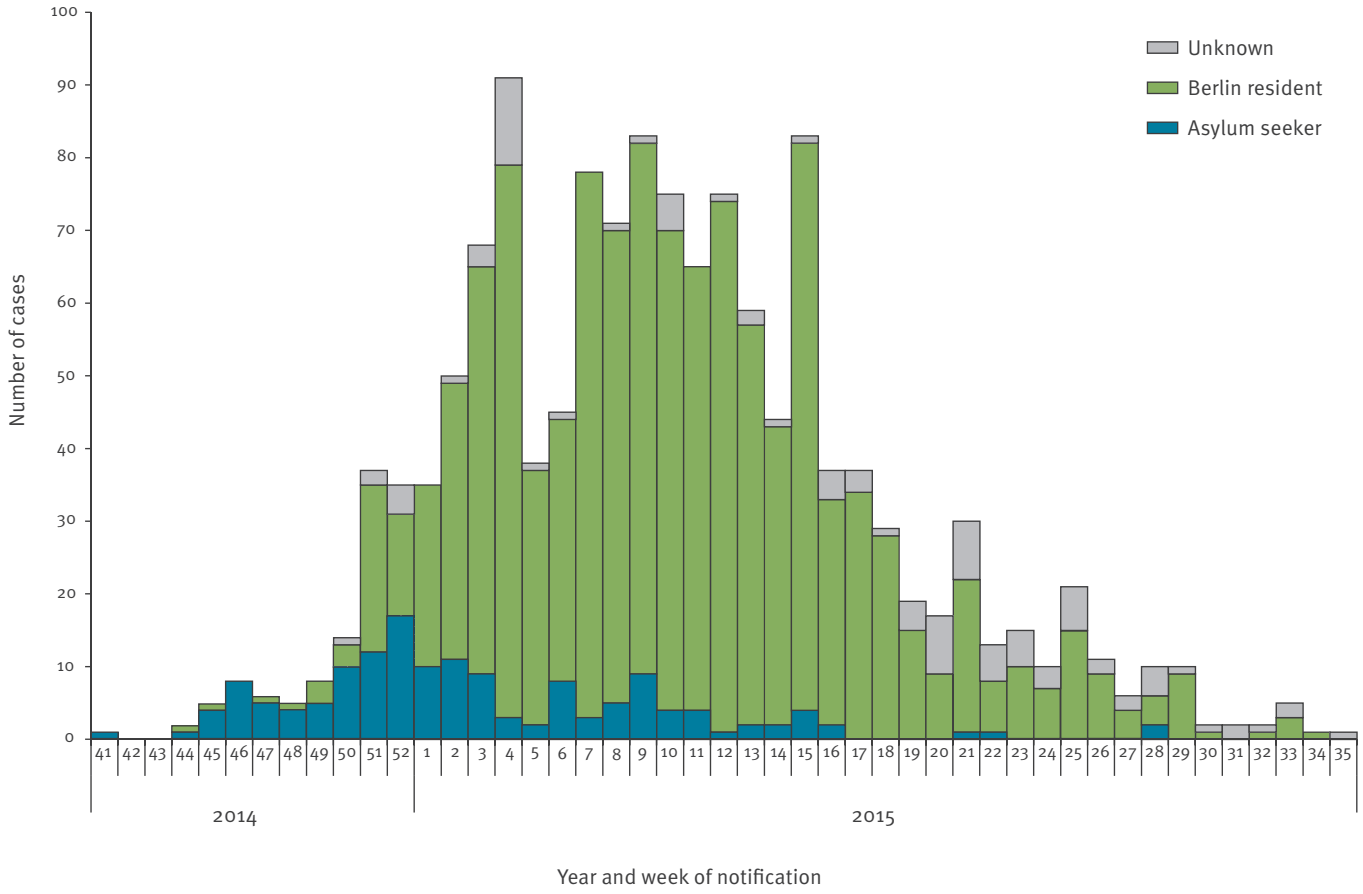
Measles is a highly communicable viral disease causing substantial morbidity and mortality globally, mostly in low-income countries [1]. Vaccination can safely and effectively prevent measles disease and measles virus (MV)-induced immunosuppression, thereby also preventing all-cause secondary infectious diseases [2]. The World Health Organization (WHO) has targeted measles and rubella for Regional elimination, and Germany has committed to this goal [3]. The key strategy for elimination is to achieve and sustain a population coverage of ≥ 95% with two doses of a MV-containing vaccine [4,5]. Thus far, elimination has only been reached in the Americas [6,7]. The WHO European Region failed to achieve the target date for elimination in 2015 [8].

In Germany, immunisation is voluntary and the German Standing Committee on Vaccination (STIKO) recommends routine administration of two doses of measles vaccine, at 11–14 and 15–23 months of age [9]. Although vaccine coverage for measles has increased substantially in children at school entry (5- to 6-year-olds) in the last decade, it is still below the 95% target (93% in Germany for two doses of measles in 2014; 92% in Berlin) [10]. Since 2010, STIKO has additionally recommended measles vaccination for adults born after 1970 with incomplete or unknown vaccination status [11].

The European Union has recently experienced a very large influx of asylum seekers with more than 600,000 and 1.3 million registered first-time applicants in 2014 and 2015, respectively [12]; Germany had the largest number of applicants. In Berlin, a city with 3.5 million inhabitants [13], 12,079 asylum seekers (12% from Bosnia and Herzegovina) were registered in 2014. This number more than tripled in 2015 (n = 44,615), with a steep increase in the second half of the year. Refugees and asylum seekers (both hereafter referred to as 'asylum seekers') should have non-discriminatory, equitable access to healthcare services, including vaccines, irrespective of their legal status [14-17]. Their right to receive vaccinations in Germany is legally anchored in the Asylum Seekers' Benefit Act [18]. Asylum seekers are accommodated in initial reception centres or collective accommodation centres (both hereafter

Figure 1

Cases by reporting week and residency status in a large outbreak of measles in Berlin, October 2014–August 2015 (n = 1,344)



referred to as 'asylum seeker homes') upon arrival in Germany. The national recommendation for post-exposure intervention in any community home, including asylum seeker homes, is to vaccinate all persons older than 9 months of age within 72 h after contact with a measles case [11].

Measles has been notifiable in Germany since 2001. From 2004 to 2013, the annual incidence per million population ranged from 1.5 to 28 without a discernible secular downward trend. During that time, the annual measles incidence was highest in Berlin, driven, in part, by a large outbreak with almost 500 reported cases in 2013. In October 2014, measles cases started to accumulate again in Berlin. Initially, most cases were in asylum seekers. We enhanced epidemiological surveillance of measles, evaluated post-exposure vaccination in homes for asylum seekers, and performed molecular surveillance of MV circulation. This report describes the epidemiological and molecular characteristics of this outbreak.

Methods

Data sources

Notification database

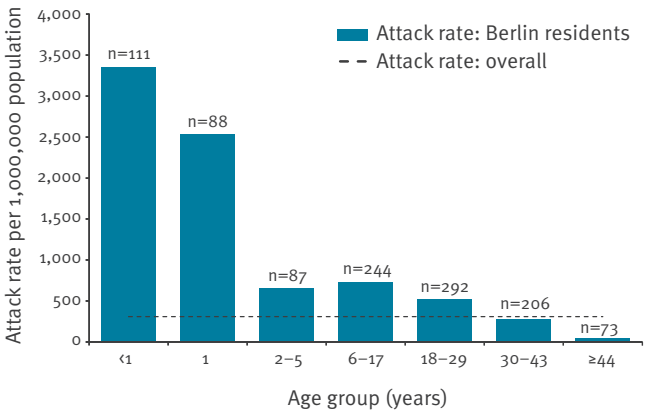
In Germany, clinical suspicion or diagnosis of measles, measles-related death and laboratory detection of measles infection are notifiable to the Local Public Health Authority (LPHA). The surveillance case definition requires that the patient have fever, maculopapular rash and one of the following: cough, coryza, Koplik spots or conjunctivitis, or an epidemiological link to a person with laboratory-confirmed measles infection. Laboratory confirmation was defined as detecting MV nucleic acid by PCR, or MV-specific IgM antibodies or a significant increase in anti-MV IgG. Cases were transmitted to the State Office for Health and Social Affairs (SOHSA) and from there to the federal level public health institute.

Additional case information

We enhanced epidemiological surveillance of measles cases by requesting that LPHAs systematically collect additional information, including residency status (asylum seeker (Y/N)), nationality, whereabouts in the two weeks before symptom onset and duration of stay in

Figure 2

Attack rates per 1,000,000 by age group among the resident population in a large outbreak of measles in Berlin, October 2014–August 2015



Germany. Information was recorded in a specifically designed Excel sheet and sent to SOHSA.

Molecular data on MV

The National Reference Centre for Measles, Mumps, Rubella (NRC MMR) at the Robert Koch Institute, Berlin, determined the MV genotype in all clinical samples confirmed by detection of viral RNA that were collected in Berlin during the outbreak period. The 'distinct sequence identifier' representing each MV sequence variant was determined and compared using the global WHO Measles Nucleotide Surveillance (MeaNS) database [19]. The phylogenetic tree was constructed using the neighbour-joining algorithm and the p-distance method as implemented in MEGA 7 [20].

For measles cases, we merged information from the notification database with additional case information and molecular data on MV by using the case identifier of the notification system or by date of onset, age, and sex (for molecular data from the NRC MMR).

Epidemiological investigation

We defined an outbreak case as illness in a person notified with measles in Berlin from 6 October 2014 (week 41) until 30 August 2015 (week 35), if the illness fulfilled the surveillance case definition and was not imported (except for the index patient), and the isolate, if genotyped, belonged to genotype D8. A case was considered to be imported if the patient was abroad during the 7 to 18 days before symptom onset.

We conducted a descriptive analysis of case characteristics by calculating frequencies and proportions or median values and interquartile ranges (IQR) as appropriate. Attack rates per million population, by age group and district, were computed for Berlin resident cases using 2013 population data from the Statistical Office for Berlin-Brandenburg.

Evaluation of post-exposure vaccinations in asylum seeker homes

We evaluated timeliness and completeness of post-exposure vaccinations in asylum seeker homes between October 2014 and February 2015. Date of symptom onset and notification of the first case was extracted from the notification system, details on the intervention (e.g. date, number of (eligible) asylum seekers vaccinated/registered) were collected by LPHAs and collated by the SOHSA. We computed median values and IQR of time intervals characterising the timeliness of the intervention. Completeness was assessed only in post-exposure vaccinations targeting all asylum seekers, which excluded homes that had closed living units and interventions restricted to specific vulnerable subgroups, e.g. children or husbands of pregnant women. It was computed as the number of vaccinated asylum seekers divided by the total number of (eligible) registered asylum seekers. Persons older than 9 months of age who were not pregnant and had no clinical signs compatible with measles and no written documentation of two measles vaccinations were eligible.

Molecular investigation

Suspected measles cases were confirmed in private or hospital laboratories or in the NRC MMR using tests for detection of anti-MV IgM in serum. Alternatively, MV RNA was detected in throat swabs, oral fluid or urine by an accredited PCR test conducted in the NRC MMR. Detected MV was genotyped by sequencing the 450 nucleotides (nt) coding for the C-terminal 150 amino acids of the nucleoprotein and phylogenetic analysis as recommended by the WHO [21]. Representative MV sequence data were submitted to the MeaNS database and to GenBank [19].

Results

Description of the outbreak

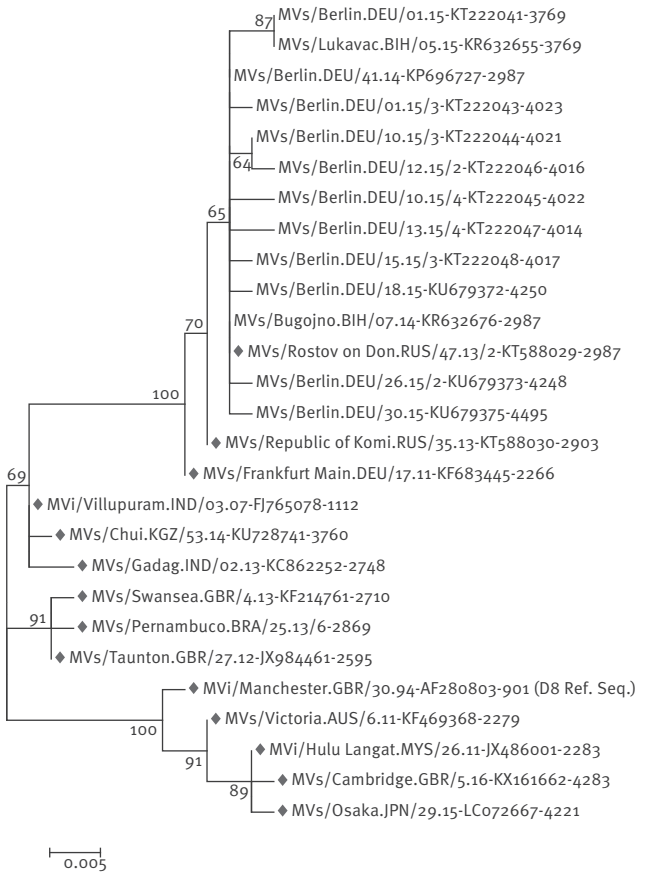
Of 1,359 cases notified during the outbreak period, 15 were considered unrelated to the outbreak; six because of genotypes other than D8 and nine because infection was considered imported, among them three asylum seekers (all from Bosnia and Herzegovina). Thus, 1,344 outbreak cases were ascertained, of which 943 (70%) were laboratory-confirmed (Table).

Median age of case patients was 17 years (IQR: 4–29 years), 737 (55%) were male, 345 (26%) were hospitalised and a 1-year-old child died of measles. In almost two thirds of cases (64%, n = 864), no link to another measles case was recorded by the LPHAs. Of those with available information (n = 1,258), 86% (n = 1,086) were not vaccinated against measles, 101 (8%) were vaccinated once, 42 (3%) twice and one case three times (0.2%) before onset of illness (for 28 (2%) the number of vaccinations was not recorded).

The index case was a 5-year-old child who travelled with their family from Bosnia and Herzegovina by bus

Figure 3

Phylogenetic relationship between the measles virus sequence variants detected in a large outbreak of measles in Berlin, August 2014–October 2015 (n = 351), a large outbreak in Bosnia and Herzegovina (2014–2015) and the World Health Organization Reference and Named Strains for measles virus genotype D8 (marked in grey)



The World Health Organization name, the GenBank accession number (if available) and the 'distinct sequence identifier' used in MeaNS are given for each sequence variant. The phylogenetic analysis is based on the 450-nt sequence encoding the C-terminus of the measles virus N protein. The tree was constructed using the Neighbour-Joining algorithm and the p-distance method as implemented in MEGA7 [20]. Bootstrap values (1,000 replicates) are shown next to the branches. The scale bar indicates a deviation of 5 nt per 1,000 nt sequence.

to Berlin (a ca 24 h drive) in early October 2014. The child had fever but no rash upon arrival. In the following months, cases occurred predominantly among asylum seekers (Figure 1).

During the outbreak, 146 (11%) cases occurred in asylum seekers (Table), most of them in children (median age: 5 years, IQR 2–18 years). Overall, 69 (47%) came from Bosnia and Herzegovina (in 2014: 41/65 cases, 63%), 41 (28%) from Serbia, eight (6%) from Syria, the remaining 20% came from 14 different countries. Measles cases occurred in 35 homes for asylum seekers, located in all Berlin districts, with a median of two cases per asylum seeker home (IQR: 1–5).

By year's end, cases started to accumulate in the Berlin resident population, with a peak in March 2015 (Figure 1). Cases among Berlin residents (n = 1,101) occurred in all districts, but the attack rate varied across the 12 districts of Berlin by a factor of almost 3.5 (highest in Neukölln: 546/1,000,000 population, lowest in Steglitz-Zehlendorf: 160/1,000,000 population (city-wide incidence: 309/1,000,000 population). Of the Berlin resident cases, almost one third (n = 349, 32%) were linked to other cases in 132 clusters (median number of cases: 2, IQR: 2–3), mostly to household members (253 cases in 106 clusters), meaning that no long transmission chains or sub-outbreaks were observed. Attack rate was highest among < 1 year olds (3,334/1,000,000, Figure 2) followed by 1 year-olds (2,538/1,000,000, which together accounted for 18% of Berlin's resident cases. The majority of cases were in adults (n = 571, 52%), most of whom (498/571, 87%) were born after 1970.

Evaluation of post-exposure vaccinations in asylum seeker homes

In the study period, cases were ascertained in 32 asylum seeker homes. Case-patients' median age in clusters in asylum seeker homes was 4 years (IQR: 1–18.5 years). Of 32 asylum seeker homes, we received no detailed information for seven homes, and in a further seven homes no post-exposure intervention was performed for different reasons (e.g. lack of resources). In the remaining 18 homes, post-exposure vaccinations were conducted with a median time interval from symptom onset of the first case to vaccination of 7.5 days (IQR: 6–10 days); in three homes vaccination occurred within the recommended 72 h after detection of measles with no further cases notified during the following 18 days. In the remaining 15 homes, 16 cases were notified during this time period. Median time interval between (i) symptom onset of the first case and notification and (ii) notification and post-exposure vaccination was 4 days (IQR: 3–8 days) and 2 days (IQR: 0–6 days), respectively.

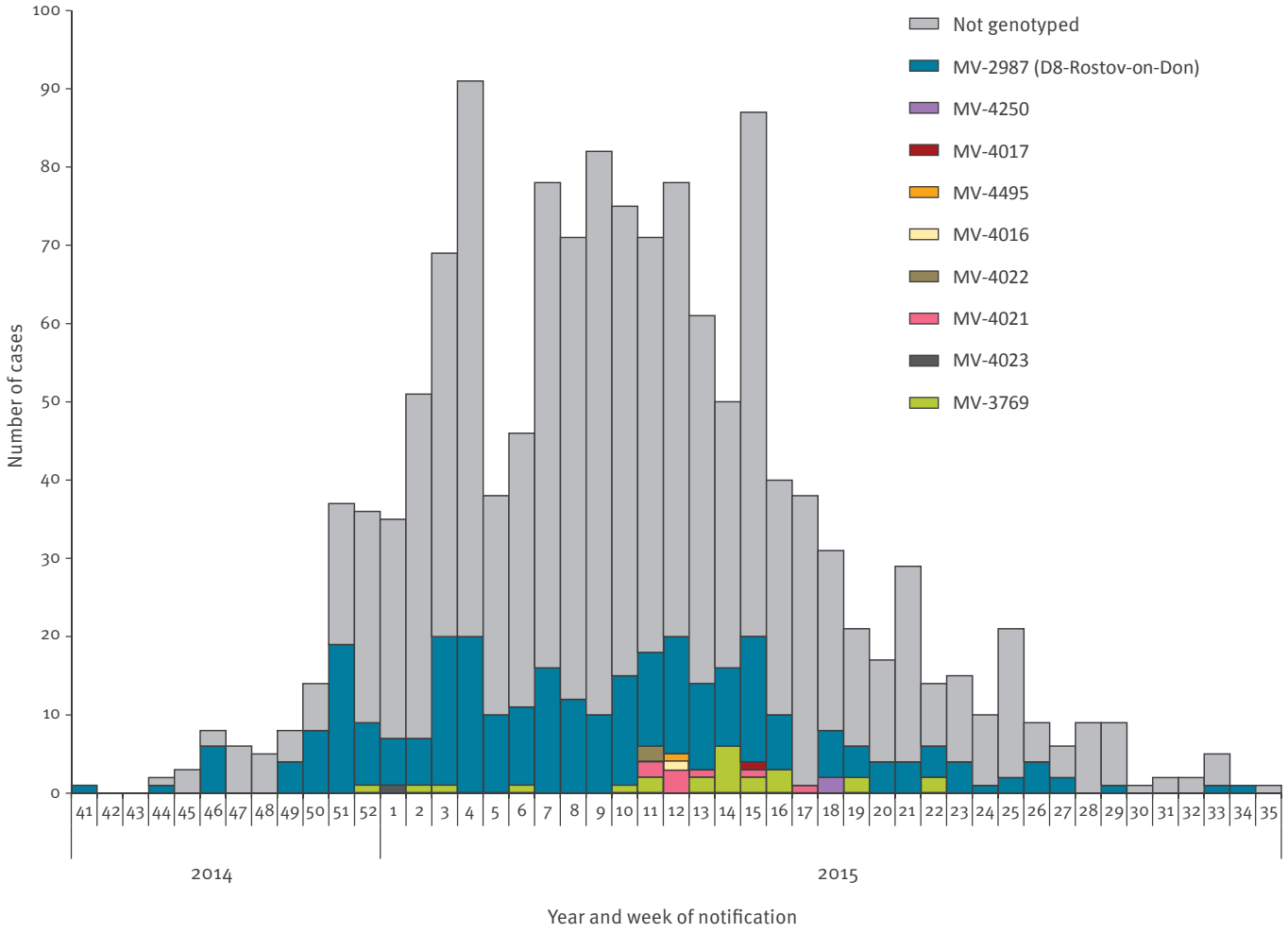
In the eight asylum seeker homes where vaccination was offered to all inhabitants and denominator data were available, 1,133/2,390 (47%) were reached. In five of these homes, eligibility criteria for vaccination were also recorded; there, 706/1,344 (53%) were reached.

Molecular investigation

During the outbreak period, the NRC received samples from 587 suspected cases. MV genome was detected in samples from 415 laboratory-confirmed cases and the MV genotype was determined for 359 cases of which 354 showed a wild-type virus. Of the 351 cases associated with genotype D8, 306 showed the predominant sequence variant Named Strain MVs/Rostov on Don. RUS/47.13/2, hereafter referred to as 'D8-Rostov-Don'. This variant was detected in Berlin during the entire outbreak period and had previously been found during a large outbreak in Bosnia and Herzegovina (February 2014 to April 2015). Forty-five cases had variants

Figure 4

Measles virus sequence variants (given by the 'distinct sequence identifier') by reporting week of cases in a large outbreak in Berlin, October 2014–August 2015 (n = 322)



deviating in the 450 nt region by 1–2 nt from D8-Rostov-Don (10 variants, Figure 3) [22]. One variant detected in Berlin from December 2014 to May 2015 (MVs/Berlin.DEU/01.15) was also found in the course of the aforementioned outbreak (MVs/Lukavac.BIH/05.15) [22].

We were able to match 322 laboratory-confirmed cases with MV genotype D8 to notified outbreak cases (254 Berlin residents and 46 asylum seekers and in 22 with unknown residency status), of which 282 (88%) had the variant D8-Rostov-Don and 40 (12%) had a 1–2 nt deviating variant (Figure 4), 11 asylum seekers, 28 Berlin residents, one case with unknown residency status. None of these cases had a travel history during their period of infection. Of these 40 cases, 24 shared an identical variant (MVs/Lukavac.BIH/05.15). The first of these cases had a symptom onset on 20 December 2014 and the last on 21 May 2015.

Discussion

In this large outbreak, MV was likely introduced by and initially spread among asylum seekers before affecting the resident population of Berlin, which ultimately accounted for the vast majority of cases. Among Berlin's resident population, all age groups eligible for vaccination were affected, with the highest attack rate in children under 2 years of age. More than half of the cases were in adults, most of whom were born after 1970, the age group for which STIKO recommends measles vaccination if vaccination history is incomplete or unknown. Interventions of LPHAs in homes for asylum seekers were mostly outside the recommended 72 h time window, reached only half of the potential contacts and apparently did not halt the outbreak. Continuous genotyping throughout the outbreak period demonstrated almost endemic (i.e. ≥12 months) transmission of the predominant variant D8-Rostov–Don in Berlin.

Several lines of evidence indicate that measles was introduced by and initially spread among asylum seekers: firstly, the index case occurred in an asylum seeker who had symptoms upon arrival in Berlin, after travelling from Bosnia and Herzegovina where a large measles outbreak was ongoing at that time [22]. Subsequent cases occurred predominantly among asylum seekers (often from Bosnia and Herzegovina) that

Table

Case characteristics in a large outbreak of measles in Berlin, October 2014–August 2015 (n = 1,344)

Characteristics	Berlin resident		Asylum seeker		Unknown		Total	
	n	%	n	%	n	%	n	%
Number of cases	1,101	100	146	100	97	100	1,344	100
Male	612	55.6	76	52.0	49	50.5	737	54.8
Laboratory-confirmed	777	70.6	99	67.8	67	69.1	943	70.2
In clusters	349	31.7	107	73.3	24	24.7	480	35.7
Unvaccinated [a]	888	85.2	127	94.1	71	87.6	1,086	86.3
Hospitalised [b]	265	24.1	35	24.0	45	46.9	345	25.7
Death	1	0.1	0	0	0	0	1	0.1
	Median	**IQR**	**Median**	**IQR**	**Median**	**IQR**	**Median**	**IQR**
Age (years)	18	5–30	5	2–18	21	9–29	17	4–29

IQR: interquartile range.

[a] Information was available for 1,258 cases on vaccination status; 1,042 Berlin residents, 135 asylum seekers, 81 unknown.

[b] Information was available for 1,342 on hospitalisation status; 1,100 Berlin residents, 146 asylum-seekers, 96 unknown.

acquired infection in Berlin. Secondly, genotyping data indicate sequence identity of MV isolated from the index case, the large majority of the following cases in Berlin, and cases of the concurrent outbreak in Bosnia and Herzegovina. Thirdly, despite of continuous monitoring of circulating MV variants, the main variant D8-Rostov-Don and closely related variants had never been found in Berlin before.

Population immunity in Berlin was too low to prevent city-wide measles spread across all age groups, equating to insufficient vaccine coverage. School-entry examinations document that coverage with two doses of a MV-containing vaccine has been consistently below 95% in Berlin, although there has been a strong increase over the past 15 years (from 21% in 1998–2001 to 92% in 2014) [10,23]. Age-dependent differences in vaccine coverage were observed in a national population survey from 2008 to 2011; coverage (1 dose) was lower in older age groups (80% in 18–29 year olds; 47% in 30–39 year olds) [24]. Taken together, population immunity likely decreases in Germany with increasing age, until age groups born before 1970.

The majority of cases were in adults. Of note, there is a clear shift towards adult measles cases in German notification data (from 11% in 2003 to 43% in 2013) [25], in the absence of a noticeable downward trend in measles notification rates over the past 10 years in Germany. Furthermore, under-reporting of measles in Germany is highest in adults [26]. Consequently, their involvement in this outbreak (and in measles epidemiology in Germany in general) can be assumed to be disproportionately underestimated and the difference in attack rates between adults and children in this outbreak is likely offset, at least partially. Interestingly, standard inquiries of cases in this outbreak by LPHAs revealed that adults tended to be ignorant of their vaccination status rather than sceptical of vaccines, suggesting they could be successfully targeted in catch-up campaigns (not yet conducted in Berlin or elsewhere in Germany) [27].

Post-exposure vaccination by LPHAs to prevent onward transmission in homes for asylum seekers were not always conducted and, where conducted, reached only half of the potential contacts. Additionally, most (83%) vaccination measures occurred after the recommended 72 h window, mainly because LPHAs were not notified in a timely fashion (vaccinating after 72 h may still be useful, e.g. to prevent tertiary cases). We did not systematically assess the reasons for delayed case notification. Anecdotally, not all cases sought medical attention immediately and sometimes the first notified case was initially misdiagnosed with a disease other than measles. Furthermore, the large number of asylum seekers in some homes often exceeded the response capacity of the LPHAs. Taken together, timely vaccination of all contacts proved challenging in these settings where LPHA often became aware of measles too late, potential contacts were often difficult to reach and large in number, and language barriers complicated intervention logistics. Of note, clusters occurred in many homes for asylum seekers (mostly involving children), but were small in size (median of two cases). This indicates a fairly high vaccination coverage among inhabitants as has been shown for migrating populations in other settings, an effect of post-exposure vaccinations, or both [28,29]. Targeting, or at least prioritising, vulnerable groups (e.g. children) in post-exposure vaccinations in (mass) asylum seeker homes should be considered, particularly in crisis situations.

It remains unclear why the outbreak became so large and long-lasting. Frequent introduction of the outbreak virus by different asylum seekers may seem an obvious explanation, but the limited available epidemiological and molecular information lends little support

to this hypothesis. Only three measles cases among asylum seekers (all from Bosnia and Herzegovina) were considered imported. Yet, under-ascertainment of measles cases is likely, particularly at the start of the outbreak (e.g. there is a gap of › 3 weeks in onset dates between the index and subsequent cases). The identification of various outbreak variants is not necessarily indicative of imported MV. All variants were closely related to the main outbreak variant D8-Rostov-Don. Most were detected only once or over a short period, and their occurrence scattered around the (virus-number) peak of the outbreak, which is compatible with random mutations of the virus. Furthermore, detection of a variant probably descending from the outbreak main variant has previously been observed in a large outbreak, in which multiple importations were deemed unlikely [30]. In keeping with that pattern, none of the (matched) cases infected with an outbreak variant deviating from the main variant were imported. However, the predominant and the second most variant identified in Berlin were also detected in the outbreak in Bosnia and Herzegovina, indicating at least two virological links to the parallel outbreak. Notwithstanding, our molecular analysis was restricted to 450 nt of the MV N gene, and therefore does not allow the monitoring of sequence variation in other regions of the viral genome. Establishing whole genome sequencing in outbreak situations across Europe might provide more detailed information. More important, however, is a continuous monitoring of virus variants during the whole outbreak period, especially when considering increasing population movement in recent years.

Enhancing the surveillance of notified measles cases, including routinely collecting information on residency status, and MV genotyping, were pivotal in understanding the epidemiology of this outbreak. Since October 2015, residency status and accompanying information have been routinely collected on all notifiable diseases in Germany. Although the data need to be interpreted with caution, they indicate that most notified infectious diseases in asylum seekers are vaccine-preventable (predominantly varicella zoster infections), and most are acquired within Germany (data not shown). This underlines the vulnerability of this group for vaccine-preventable diseases.

No city-wide system for offering vaccinations to asylum seekers was in place during the outbreak period. Since September 2015, Berlin has offered immunisation with MV- (and polio-) containing vaccines, as recommended by WHO, the European Centre for Disease Prevention and Control and STIKO, to all asylum seekers in Berlin [31,32]. Coupled with a brief medical examination, this has become an integral part of the asylum seekers' registration process since March 2016 in Berlin. Notwithstanding, there remains a continued risk of importation of MV as many residents travel and many travellers arrive. In 2014, 12.4 million people visited Berlin, of which 4.8 million came from abroad (one fifth of all foreign visitors to Germany) [33]. In conjunction with insufficient population immunity, eliminating measles in Berlin is likely to remain a distant prospect in the absence of supplementary immunisation activities.

Conclusion

This outbreak exemplifies why, in addition to ethical and legal grounds, asylum seekers should be timely offered vaccination against measles: to protect them. In addition, catch-up campaigns to close immunisation gaps, particularly in adults, are urgently needed in Berlin's resident population. Surveillance of infectious diseases should routinely collect information on residency status to be able to assess and quickly respond to infectious disease risks in asylum seekers. MV genotyping throughout the outbreak period demonstrated continuous circulation of variant D8-Rostov-Don for almost 11 months in Berlin.

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Conflict of interest

None declared.

Authors' contributions

DW contributed to data collection, data analysis, data interpretation and drafting the manuscript.

AH contributed to literature search, data collection, data analysis, data interpretation, creating graphics and drafting the manuscript.

AM contributed to molecular-biological investigation. SS contributed to molecular-biological investigation and creating graphics.

DS contributed to data collection and data interpretation.

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Research article

The effectiveness of influenza vaccination in preventing hospitalisations of elderly individuals in two influenza seasons: a multicentre case–control study, Spain, 2013/14 and 2014/15

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Influenza vaccination may limit the impact of influenza in the community. The aim of this study was to assess the effectiveness of influenza vaccination in preventing hospitalisation in individuals aged ≥ 65 years in Spain. A multicentre case–control study was conducted in 20 Spanish hospitals during 2013/14 and 2014/15. Patients aged ≥ 65 years who were hospitalised with laboratory-confirmed influenza were matched with controls according to sex, age and date of hospitalisation. Adjusted vaccine effectiveness (VE) was calculated by multivariate conditional logistic regression. A total of 728 cases and 1,826 matched controls were included in the study. Overall VE was 36% (95% confidence interval (CI): 22–47). VE was 51% (95% CI: 15–71) in patients without high-risk medical conditions and 30% (95% CI: 14–44) in patients with them. VE was 39% (95% CI: 20–53) in patients aged 65–79 years and 34% (95% CI: 11–51) in patients aged ≥ 80 years, and was greater against the influenza A(H1N1)pdm09 subtype than the A(H3N2) subtype. Influenza vaccination was effective in preventing hospitalisations of elderly individuals.

Introduction

Influenza is an acute illness caused by influenza viruses. During seasonal epidemics, large numbers of influenza infections occur in all age groups. In most individuals, influenza is a self-limiting illness, but serious secondary complications appear in some of those infected with the influenza viruses. Influenza virus infection-related morbidity and mortality is a serious human health concern worldwide, affecting health of populations and economies worldwide. The illness may result in hospitalisation, overwhelming hospitals and causing excess influenza health-related deaths [1]. Worldwide, annual epidemics are estimated to result in ca 3 to 5 million cases of severe illness and ca 250,000 to 500,000 deaths [2]. Individuals who are elderly, especially those with comorbidities, are particularly at risk for influenza-related complications and frequently require hospitalisation. In an American study carried out in the 2005/06 through 2013/14 seasons, 89% of all influenza-associated deaths were in people aged ≥ 65 years [3]. A recent French study estimated that 11% of all-cause deaths in elderly individuals during the influenza season were attributable to influenza [4]. However, mortality is just the tip of the iceberg in terms of disease and the economic burden, and hospitalisation is also an important outcome that should be considered [5].

The capacity of influenza viruses to undergo gradual antigenic change in their surface antigens is a

Table 1

Distribution of influenza cases and controls aged ≥ 65 years according to demographic variables, medical conditions and history of vaccination, Spain, influenza seasons 2013/14 and 2014/1

Characteristics	Cases (n = 728)		Controls (n = 1,826)		Crude OR	95% CI	p value
	n	%	n	%			
Age group							
65–79 years	411	56.5	1,016	57.0	Ref	Ref	0.50
≥ 80 years	317	43.5	810	43.0	0.89	0.64–1.24	
Sex							
Female	343	47.1	884	48.4	NA	NA	NA
Male	385	52.9	942	51.6	NA	NA	NA
Marital status							
Married/cohabiting	450	61.9	1,020	56.0	Ref	Ref	Ref
Single	39	5.4	145	8.0	0.57	0.39–0.83	0.004
Widowed	217	29.8	615	33.8	0.76	0.61–0.95	0.02
Separated/divorced	21	2.9	42	2.3	1.17	0.68–2.00	0.57
Educational level							
Without or primary	560	77.0	1,349	74.9	Ref	Ref	0.07
Secondary or higher	167	23.0	453	25.1	0.81	0.64–1.01	
Barthel Index[a]							
0–90[a]	276	37.9	796	43.6	0.79	0.64–0.96	0.02
>90[a]	452	62.1	1,028	56.4	Ref	Ref	
Smoking status							
No smoker	383	52.6	1,057	57.9	Ref	Ref	0.01
Smoker/ex-smoker	345	47.4	769	42.1	1.39	1.09–1.77	
High alcohol consumption[b]							
Yes	16	2.2	53	2.9	0.77	0.43–1.38	0.38
No	712	97.8	1,772	97.1	Ref	Ref	
Number of hospital visits during the past year							
0–2	403	56.1	916	50.6	Ref	Ref	0.05
≥ 3	316	43.9	896	49.4	0.82	0.67–1.00	
High-risk medical conditions							
No	104	14.3	386	21.1	Ref	Ref	<0.001
Yes	624	85.7	1,440	78.9	1.73	1.35–2.22	
Current-season influenza vaccine received							
Yes	359	49.3	1,053	57.7	0.73	0.61–0.87	<0.001
No	369	50.7	773	42.3	Ref	Ref	
Previous-season influenza vaccine received							
Yes	376	51.6	1,054	57.7	0.78	0.66–0.93	0.005
No	352	48.4	772	42.3	Ref	Ref	
Pneumococcal vaccine received							
Yes	372	51.1	836	45.8	1.20	0.99–1.46	0.06
No	356	48.9	990	54.2	Ref	Ref	

CI: confidence interval; NA: not applicable; OR: odds ratio; Ref: reference group for comparison.

[a] The Barthel Index is a measurement of limitations in activity, ranging from 0 (complete dependence) to 100 (complete independence).

[b] High alcohol consumption defined as >40 g/day for men and >24 g/day for women.

challenge for vaccination against seasonal influenza. Annual administration of the seasonal influenza vaccine, especially in those known to be at high risk of serious complications as a result of influenza, is the focus of current efforts to reduce the disease impact [1]. In the 2013/14 season, the trivalent inactivated vaccine administered in Spain and in all the northern hemisphere, contained an A/California/7/2009(H1N1) pdm-09-like virus, an A(H3N2) virus antigenically similar to the cell-propagated prototype virus A/Victoria/361/2011 and a B/Massachusetts/2/2011-like virus. For the 2014/15 season, the vaccine composition only changed the A/Victoria/361/2011 component

Table 2

Distribution of influenza cases and controls aged ≥ 65 years according to comorbidities, Spain, influenza seasons 2013/14 and 2014/15

Characteristics	Cases (n = 728)		Controls (n = 1,826)		Crude OR	95% CI	p value
	n	%	n	%			
High-risk medical conditions							
COPD	194	26.6	218	11.9	3.03	2.37–3.88	< 0.001
Chronic respiratory failure	119	16.3	208	11.4	1.64	1.26–2.14	< 0.001
Pneumonia past 2 years	91	12.5	104	5.7	2.40	1.77–3.26	< 0.001
Other lung disease	238	32.7	380	20.8	1.88	1.54–2.30	< 0.001
Cardiovascular disease	224	30.8	651	35.7	0.84	0.68–1.03	0.09
Diabetes mellitus	235	32.3	666	36.5	0.89	0.74–1.07	0.21
Renal failure with hemodialysis	16	2.2	31	1.7	1.27	0.68–2.37	0.46
Hemaglobinopathy or anaemia	89	12.2	306	16.8	0.68	0.52–0.88	0.004
AIDS	1	0.1	2	0.1	1.30	0.12–14.51	0.83
Asymptomatic HIV infection	3	0.4	1	0.1	9.00	0.94–86.52	0.06
Neurological disease	51	7.0	136	7.4	0.93	0.64–1.33	0.93
Obesity (BMI ≥ 30 kg/m²)	174	23.9	370	20.3	1.25	1.01–1.56	0.04
Non high-risk medical conditions							
Solid organ neoplasia	103	14.1	348	19.1	0.67	0.52–0.85	0.001
Haematologic neoplasia	39	5.4	40	2.2	2.57	1.62–4.07	< 0.001
Transplantation	22	3.0	10	0.5	5.52	2.52–12.09	< 0.001
Immunosuppressive treatment	35	4.8	67	3.7	1.35	0.87–2.08	0.18
Oral corticosteroid therapy	44	6.0	43	2.4	2.54	1.62–3.97	< 0.001
Asplenia	2	0.3	8	0.4	0.66	0.14–3.11	0.60
Renal failure without hemodialysis	135	18.5	341	18.7	1.01	0.80–1.26	0.94
Nephrotic syndrome	7	1.0	14	0.8	1.14	0.45–2.88	0.78
Autoimmune disease	47	6.5	96	5.3	1.38	0.94–2.03	0.10
Chronic liver disease	28	3.8	95	5.2	0.76	0.49–1.18	0.22
Cognitive dysfunction	76	10.4	205	11.2	0.92	0.68–1.23	0.92
Neuromuscular disease	24	3.3	53	2.9	1.16	0.70–1.93	0.55
Convulsions	8	1.1	23	1.3	0.84	0.37–1.92	0.69

BMI: body mass index; CI: confidence interval; COPD: chronic obstructive pulmonary disease; OR: odds ratio.

to the A/Texas/50/2012 component, an antigenically similar virus.

Various factors affect influenza vaccine effectiveness (VE). One main factor is the antigenic similarity or dissimilarity between circulating strains and vaccine strains: VE decreases with increasing antigenic distance between vaccine components and circulating strains [6]. There was no mismatch in 2013/14 for the A(H1N1)pdm09 and A(H3N2) components but in 2014/15, some degree of mismatch for the A(H3N2) circulating strain was observed [7,8]. Another factor is the influenza illness rate, which may vary substantially from year to year; in years with low rates, the power of some studies to detect significant VE may be compromised [9]. Therefore, studies including more than one season are recommended in order to estimate VE.

The aim of this study was to assess the effectiveness of influenza vaccination in preventing hospitalisation due to laboratory-confirmed influenza in individuals aged ≥ 65 years during two influenza seasons (2013/14 and 2014/15) in Spain.

Methods

Study design

We carried out a multicentre case–control study in 20 major hospitals from seven of 17 Spanish regions (Andalusia, the Basque Country, Catalonia, Castile and Leon, Madrid, Navarra and Valencian Community), covering 1,444,688 individuals aged ≥ 65 years and representing 16.8% of the Spanish population in this age group. Cases and corresponding controls admitted to participating hospitals between December 2013 and March 2015 were recruited.

Selection of cases and controls

We selected patients aged ≥ 65 years who were hospitalised for at least 24 hours with laboratory-confirmed

Table 3

Crude and adjusted influenza vaccine effectiveness against hospitalisation because of influenza in individuals aged ≥ 65 years according to influenza season, presence or absence of high-risk medical conditions, case age and type/subtype of influenza virus, Spain, influenza seasons 2013/14 and 2014/15

Variables	Cases vaccinated/n	%	Controls vaccinated/n	%	Crude vaccine effectiveness	95% CI	p value	Adjusted vaccine effectiveness	95% CI	p value
All	359/728	49.3	1,053/1,826	57.7	27%	13–39	<0.001	36%	22–47	<0.001
2013/14 season	208/433	48.0	602/1,038	58.0	31%	13–45	0.002	37%	19–51	<0.001
2014/15 season	151/295	51.2	451/788	57.2	21%	−3 to 40	0.08	34%	10–52	0.01
Non high-risk medical conditions	42/104	40.4	159/255	62.4	54%	27–71	0.001	51%	15–71	0.01
High-risk medical conditions	317/624	50.8	894/1,571	56.9	21%	7–32	0.01	30%	14–44	<0.001
65–79 years of age	183/411	44.5	561/1,040	53.9	29%	11–44	0.003	39%	20–53	<0.001
≥ 80 years of age	176/317	55.5	492/786	62.6	24%	0–42	0.05	34%	12–51	0.01
Influenza A	334/687	48.6	991/1,717	57.7	30%	16–41	<0.001	37%	23–48	<0.001
Influenza A(H1N1) pdm09	139/325	42.8	464/823	56.4	41%	24–55	<0.001	49%	32–62	<0.001
Influenza A(H3N2)	138/256	53.9	393/652	60.3	22%	−5 to 42	0.10	26%	−3 to 47	0.08[a]
Influenza B	24/39	61.5	58/103	56.3	−35%	−187 to 36	0.43	18%	−145 to 73	0.72[b]

CI: confidence interval

[a] Statistical power: 74%.

[b] Statistical power: 10%.

(PCR, culture or immunofluorescence) influenza virus infection.

For each case, up to three matched controls from among patients aged ≥ 65 years with unplanned hospital admission due to causes other than influenza or acute respiratory disease were selected. Controls were matched with each case according to sex, age (± 3 years) and date of hospitalisation (± 10 days). They were selected from patients admitted to the internal medicine, general surgery, otorhinolaryngology, ophthalmology, dermatology or traumatology services wards. Patients referred from nursing homes and those who did not provide written informed consent were excluded.

Data collection

The following demographic variables and pre-existing medical conditions were recorded: age, sex, marital status, educational level, smoking and high alcohol consumption (› 40 g/day for men and › 24 g/day for women), number of hospital visits during the last year, the Barthel Index as a measurement of limitations in activity (ranging from 0 (complete dependence) to 100 (complete independence)), chronic obstructive pulmonary disease (COPD), chronic respiratory failure, history of pneumonia during the last two years, other lung diseases, neoplasia, transplantation, immunosuppressive treatment, asplenia, diabetes mellitus, renal failure, nephrotic syndrome, autoimmune disease, AIDS, asymptomatic HIV infection, congestive heart disease, disabling neurological disease, obesity (body mass index ≥ 30 kg/m2), chronic liver disease, haemoglobinopathy or anaemia, cognitive dysfunction, convulsions and neuromuscular disease. Information on influenza vaccination in the current and previous season, and information on pneumococcal vaccination was collected.

Cases were considered vaccinated with the current influenza vaccine or pneumococcal vaccine if they had received a dose of the vaccine ≥ 14 days before symptom onset. Controls were considered vaccinated if they had received a dose of the influenza vaccine ≥ 14 days before the onset of symptoms of the matched case. Influenza vaccination in the previous season in cases and controls was defined as administration of the seasonal influenza vaccine during the preceding influenza season.

Statistical analysis

A bivariate comparison for matched data of demographic variables and medical conditions between cases and controls was made using McNemar's test. A two-tailed distribution was assumed for all p values.

A univariate conditional logistic regression model was used to estimate the crude VE in preventing influenza hospitalisation. Propensity score (PS) analysis was used to evaluate the adjusted VE. The PS was created using a logistic regression model with influenza vaccination status as the outcome and demographic variables, medical conditions and functional status as independent variables. The PS was used as a continuous covariate in a final conditional logistic regression model.

Table 4

Crude and adjusted influenza vaccine effectiveness against hospitalisation because of influenza in individuals aged ≥ 65 years according to current and previous influenza vaccination, Spain, influenza seasons 2013/14 and 2014/15

Vaccination status	Cases (n = 728)		Controls (n = 1,826)		Crude vaccine effectiveness	95% CI	p value	Adjusted vaccine effectiveness	95% CI	p value
	n	%	n	%						
Vaccinated in current season only	52	7.1	160	8.8	35%	7–54	0.02	41%	16–59	0.004
Vaccinated in previous season only	69	9.5	161	8.8	13%	−20 to 37	0.39	24%	−6 to 45	0.11[a]
Vaccinated in both seasons	307	42.2	893	48.9	28%	13–41	0.001	42%	28–54	< 0.001
Not vaccinated	300	41.2	612	33.5	Ref	Ref	Ref	Ref	Ref	Ref

CI: confidence interval; Ref: reference group for comparison.

[a] Statistical power: 54%.

Using the formula $VE = (1 - OR) \times 100$, VE was calculated globally, by season, for the presence of high-risk medical conditions, for age groups, for type/subtype of influenza virus and for each category of vaccine exposure: vaccinated only in current season, only in prior season, in both seasons, and unvaccinated in both seasons as the reference group.

The analysis was performed using the SPSS version 23 statistical package and the R version 3.3.0 statistical software [10].

Ethical considerations

All data collected were treated as confidential, in strict observance of legislation on observational studies. The study was approved by the ethics committees of the participating hospitals. Written informed consent was obtained from all patients included in the study.

Results

A total of 728 cases and 1,826 controls were included in the study. The distribution of cases and controls according to demographic variables, medical conditions and vaccination history is shown in Table 1. A total of 359 cases (49.3%) and 1,053 controls (57.7%) had received influenza vaccination. Of the 728 cases, 433 were from the 2013/14 season and 295 were from the 2014/15 season. Of the 433 cases from the 2013/14 season, 429 (99.1%) were infected with influenza A virus (59.8% were A(H1N1)pdm09, 30.5% were A(H3N2) and 8.8% were unsubtyped), two cases were infected with influenza B virus and two cases were missing data for type and subtype. Of the 295 cases from the 2014/15 season, 258 (87.5%) were infected with influenza A virus (22.4% were A(H1N1)pdm09, 42.0% were A(H3N2) and 23.1% were unsubtyped) and 37 (12.5%) were infected with influenza B virus.

Most cases (85.7%) and controls (78.9%) had high-risk medical conditions (Table 2).

The overall adjusted VE against influenza hospitalisation in individuals aged ≥ 65 years was 36% (95% CI: 22–47), without relevant differences between seasons (34%, 95% CI: 10–52 in 2013/14 and 37%, 95% CI: 19–51 in 2014/15) (Table 3). The adjusted VE was greater, but not significantly different, in patients without high-risk medical conditions (51%, 95% CI: 15–71) and in patients aged 65–79 years (39%, 95% CI: 20–53). Adjusted VE was 37% (95% CI: 23–48) for all influenza A viruses, 49% (95% CI: 32–62) for influenza A(H1N1)pdm09 and 26% (95% CI: −3 to 47) for influenza A(H3N2). Protection against influenza B was lower (VE 18%, 95% CI: −145 to 73), but the number of cases was very low (statistical power: 10%).

Adjusted VE against hospitalisation was 41% (95% CI: 16–59) among those only vaccinated in the current season and 42% (95% CI: 28–54) among those vaccinated in both the current and previous season. VE among those only vaccinated in the previous season only was 24% (95% CI: −6 to 45) (Table 4).

Discussion

The results of this study over two seasons, one with predominant circulation of influenza A (H1N1)pdm09 and one with A(H3N2) predominance, show overall VE against hospitalisation in individuals aged ≥ 65 years was 36% (95% CI: 22–47).

Some studies investigating the prevention of influenza hospitalisation among individuals who are elderly show greater VE [11,12]. In a German study using the screening method, VE in preventing confirmed influenza hospitalisation in individuals aged ≥ 60 years varied between 62% in the 2011/12 season, when the predominant influenza virus strain was A(H3N2), and 83% in the 2010/11 season, when the predominant strain was A(H1N1)pdm09 [11]. However, these levels of VE might be an overestimate because information on comorbidities was not available to adjust them by [13]. A Spanish case–control study for the 2014/15 season, when the predominant strain was A(H3N2), using test-negative controls in 10 hospitals not included in the present study found a VE of 40% (95% CI: 13–59) in terms of preventing hospital admissions in patients 65

years of age and older [14]. A 2014 New Zealand study, also using a test-negative control design, found a VE of 21% (95% CI: −82 to 66) for influenza-related hospitalisation in patients aged ≥ 65 years [15].

An American test-negative study by Petrie et al. [16] during the 2014/15 season found a VE of 48% (95% CI: −33 to 80) in people aged ≥ 65 years, but the number of individuals included was lower than in the present study. A Chinese test-negative study in people aged › 60 years during the two seasons included in our study, but with a lower number of individuals than in our study, found a point estimate of VE of 27% (95% CI: −114 to 75) during the 2013/14 season. However, no effectiveness was observed in the 2014/15 season [17]. The possible influence of increasing age on VE has been investigated. In our study, adjusted VE against hospitalisations was 39% (95% CI: 20–53) in patients aged 65–79 years and 34% (95% CI: 12–51) in patients aged ≥ 80 years. Decreasing effectiveness has been linked to advanced age in different studies [12,18,19]. Senescence diminishes immunity to influenza infections and the response to vaccination, possibly explaining the lower VE in elderly individuals than in the general population [20].

In terms of analysing VE in older age groups, the German study by Remschmidt et al. found that the VE point estimate against laboratory-confirmed influenza was greater in individuals aged 60–69 years than in older individuals in the 2011/12 season, but the opposite was observed in the 2010/11 season [11]. More research is needed to assess this matter.

In our study, VE was 30% (95% CI: 14–44) in patients with high-risk medical conditions, which was lower than that found in patients without these conditions. Similar results were obtained by other studies [16,21]. In contrast, a 2014/15 Canadian test-negative case–control study of individuals aged ≥1 year by Skowronski et al. [22] did not find a lower age-adjusted VE in patients with comorbidities (16%, 95% CI: −28 to 44) than in patients without comorbidities (6%, 95% CI: −20 to 27). Comorbidities, like age, are strongly associated with a lack of response to vaccination [23]. In fact, one of the major mechanisms through which vaccination is thought to reduce mortality is by blunting influenza-triggered exacerbations of underlying diseases [9]. However, despite the limited VE, the benefits of vaccination may be greater in patients with comorbidities because influenza is associated with a higher risk of severe disease and death in these individuals [24].

Similar to the results of other studies of VE in elderly individuals [11,25], the present study found that VE for subtype A(H1N1)pdm09 was greater (49%, 95% CI: 32–62) than that for subtype A(H3N2) (26%, 95% CI: −3 to 47).

Small, non-significant VE differences were found according to season. In the 2013/14 season, an antigenic mismatch was observed in the B virus component but the A(H1N1)pdm09 and A(H3N2) strains circulating were analogous to the seasonal vaccine strains [7]. However, in some Spanish regions, specific mutations of A(H1N1) and A(H3N2) strains associated with low VE and outbreaks in institutions were found [26]. In the 2014/15 season, mismatched A(H3N2) strains circulated widely around the world [27], but only accounted for 60% of influenza A virus isolates in Spain [8]. This might explain why no relevant differences were found in VE in these two influenza seasons.

In our study, VE in individuals vaccinated only in the current season was similar to that of individuals vaccinated in both the current and previous seasons (41%, 95% CI: 16–59 and 42%, 95% CI: 28–54, respectively), which does not support interference between current and previous vaccination. Three 2014/15 influenza season studies carried out on populations of various ages [16,22,28] reported that vaccination in the previous and current season may diminish VE only in the current season, suggesting negative interference from prior vaccination when the antigenic distance between the vaccine and circulating strains is large but the antigenic distance between vaccine components in consecutive seasons is small [22]. The effects of the various combinations of agent-host factors involved in this phenomenon remain unclear and more research is required to determine their influence on vaccine-induced influenza virus immunity in elderly individuals. However, in agreement with Neuzil [29], we consider that the current policy of administering the influenza vaccine every year should be maintained in the meantime. As the most-vulnerable elderly individuals are those with the most advanced age because they have a higher risk of hospitalisation and death compared with healthy elderly individuals aged 65–75 years [20], seasonal influenza vaccination programs in all elderly individuals should be reinforced.

This study has strengths and limitations. Strengths of this study are the matching design, the high number of covariates recorded and the fact that the vaccination status was obtained by consulting hospital records, vaccination cards and primary health registers.

The limitations include the fact that controls were not systematically swabbed and therefore they may, theoretically, have been infected with influenza virus. However, controls were patients with unplanned admission to hospital because of causes other than influenza or acute respiratory disease, and it seems unlikely that selection bias could invalidate our results. A possible confounder is the functional status; however, we included the Barthel Index in the propensity score and therefore this limitation is reasonably controlled for. Likewise, it is important to consider the weeks with influenza activity in the analysis, but because cases and controls were matched by admission date, we believe this is unlikely to invalidate the results. Another possible limitation is that cases and controls

were recruited in 20 major hospitals, but as these hospitals cover 16.8% of the Spanish population aged ≥ 65 years we believe that the study is representative of the older Spanish population. Also, we have not collected information on patients' influenza-like illness in previous seasons, but previous episodes of influenza does not usually act as a confounding factor that needs to be controlled for in studies evaluating influenza VE [30]. Finally, the low statistical power in the investigation of VE against influenza B virus because of the very low number of cases in the two seasons studied was another limitation.

In conclusion, the results of this study show that influenza vaccination was effective in preventing hospitalisations because of influenza in individuals who are elderly. The point estimates of the adjusted VE were highest in patients without high-risk medical conditions, in patients in the 65–79 years of age group and against the influenza A(H1N1)pdm09 subtype compared with the A(H3N2) subtype, although the 95% confidence limits overlapped. Finally, we found that VE was similar between vaccination only in the current season and vaccination in both the current and the previous seasons.

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Conflict of interest

None declared.

Authors' contributions

All the authors participated in the study design, implementation and interpretation. AD, NS and DT had full access to all the study data and take responsibility for the data accuracy of the data analysis. AD and NS designed the study and drafted the report. NS conducted the statistical analysis. PG, EE, MAF, JMM, JC, ME, ST, JA and MMSV designed and supervised the study, and reviewed the draft report. The other members of the Working Group contributed to the design of the study, patient recruitment, data collection and interpretation of the results.

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