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Molecular identification of emergent GII.P17-GII.17 norovirus genotype, Romania, 2015

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The novel GII.P17-GII.17 norovirus genotype has been reported as cause of gastroenteritis outbreaks in China and Japan since the winter season 2014/15, replacing the pandemic strain GII.4 Sydney 2012. These emergent strains have also been sporadically reported on other continents than Asia. GII.P17-GII.17 isolates, similar to Kawasaki308 2015, were identified in three patients during a large outbreak of acute gastroenteritis affecting 328 people in Romania, in neighbouring localities, in 2015.

We present molecular evidence for the circulation of emergent norovirus GII.P17-GII.17 strain during an outbreak of acute gastroenteritis that occurred in Arad, a county in the western part of Romania, in 2015.

Noroviruses are among the leading causes of non-bacterial gastroenteritis worldwide [1]. Published data on circulation of noroviruses in Romania are scarce [2,3] and sequences previously obtained by our group (i.e. GenBank FR695414-FR695417) indicated circulation of genotype GII.P21-GII.2 in 2006 (unpublished data).

Origin of samples

Between 16 October and 1 December 2015, an outbreak of acute gastroenteritis was recorded in Arad, a county in the western part of Romania, bordering Hungary, with ca 400,000 inhabitants. The first cases were notified by a local hospital to Arad Public Health Department on 18 October 2015. The public health authorities further investigated the outbreak-associated cases among household contacts, in schools and hospitals. Suspected cases were defined as patients with three or more loose stools in a 24-hour period, and/or two or more episodes of vomiting in a 24-hour period. Confirmed cases were suspected cases additionally testing positive for norovirus by reverse transcription-polymerase chain reaction (RT-PCR). Three hundred twenty-eight cases (sex ratio: 0.8:1; 145 male: 183 female) were recorded across 20 small rural and urban neighbouring communities with a total number of ca 35,000 inhabitants. The patients' median age was 18 years and the average age was 27 years (standard deviation: 22; interquartile range: 10-40; age range: 0–95 years). Cases were either clustered in foci (n = 302) or sporadic (n = 26). One hundred fourteen cases were recorded in schools. The illness was mild, self-limiting, with only eight patients hospitalised. No further information was available on the reasons for hospitalisation. No fatalities were recorded. The onset was sudden and symptoms included nausea (n = 220), vomiting (n = 214), abdominal pain (n = 178), diarrhoea (n = 168), headache (n = 100), dehydration (n = 30) and fever (n = 20). The index case was not identified.

Laboratory investigation

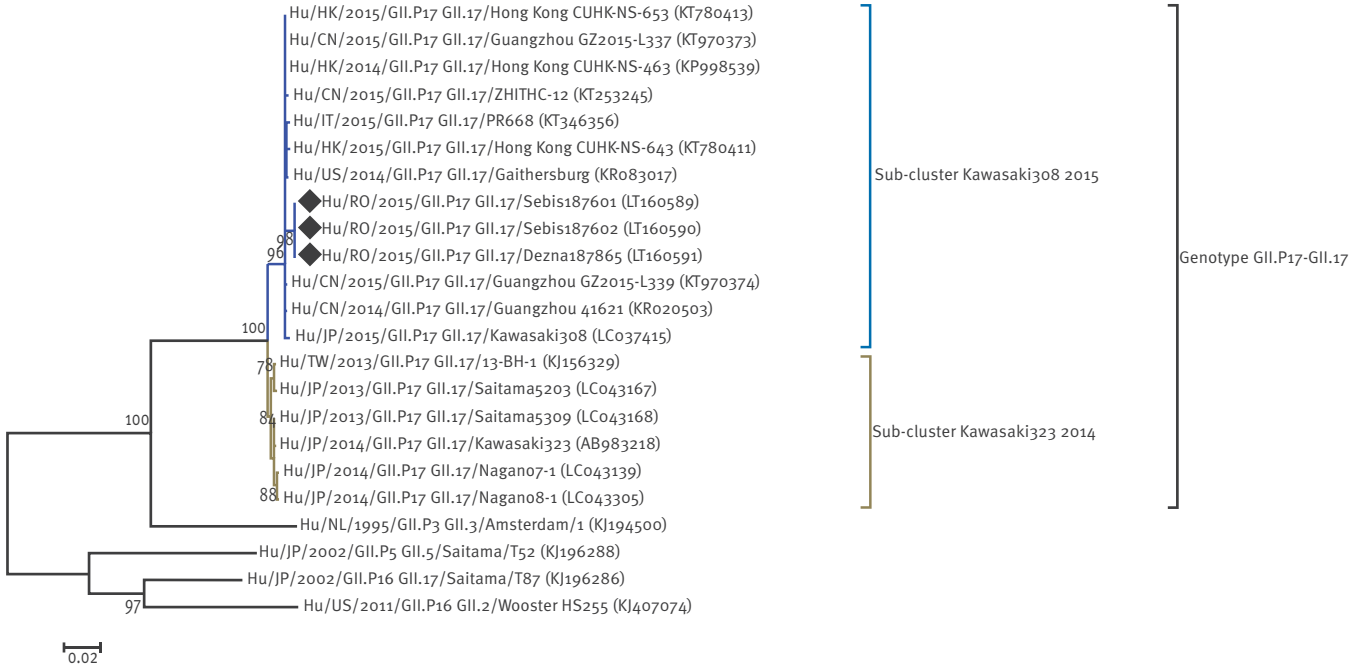
Five stool samples collected between 25 October and 7 November 2015 from patients with acute gastroenteritis were received for molecular diagnostic and genotyping at Cantacuzino National Institute of Research in Bucharest.

Viral RNA was extracted from 140 µL of PBS stool suspension (10% wt/vol) using QIAamp RNA Viral Mini Kit (Qiagen). A fragment of 1111 nt spanning ORF1-ORF2 (RdRp-VP1) junction, region recommended for norovirus typing, was amplified using primers JV12 and G2SKR [4,5].

Three out of five samples yielded a PCR product suitable for sequencing (one sample from an adult and two samples from two young children). Sequencing was performed with BigDye Terminator v3.1 (Applied Biosystems) and Norovirus Genotyping Tool (http://

Figure

Neighbour-joining tree of GII norovirus isolates based on partial RdRp-VP1 junction, outbreak of acute gastroenteritis, Romania, 2015



Black diamonds: sequences obtained in this study from three patients during an outbreak of acute gastroenteritis in Arad county, Romania, 2015.

Numbers at nodes represent the bootstrap percentages (values < 70% are not shown). The analysis was conducted on a 1067 nt sequence (nt positions 4303–5369 in isolate Kawasaki323 2014, GenBank accession number AB983218).

www.rivm.nl/mpf/norovirus/typingtool) was employed for assigning genotype [6]. The three sequences obtained were identical and the results of typing indicated genotype GII.P17-GII.17. Sequences were deposited in GenBank under the accession numbers LT160589, LT160590, and LT160591.

Phylogenetic analysis was conducted with Mega 6 software [7], neighbour-joining statistical method, Kimura 2-parameter, 1,000 bootstrap replications. Phylogenetic analysis (Figure) grouped the sequences described here in the same sub-cluster with Kawasaki308 strain, identified in Japan in 2015 [8].

Discussion

GII.17 norovirus genotype was first described in French Guiana, in 1978 [9]. As reviewed elsewhere [10], GII.17 strains have been sporadically detected in Africa, America, Asia and Europe. Strains belonging to the novel GII.P17-GII.17 norovirus genotype were detected in Korea in 2013 and have been associated with gastroenteritis outbreaks in China and Japan since 2014/15 winter season, replacing the previously dominant strain GII.4 Sydney 2012 [8,10-13]. The same genotype was identified in groundwater in Kenya, in 2012 [14]. Molecular dating analysis estimated that GII.P17-GII.17 strains have been circulating in Asia as early as 2002 [8].

In Europe, GII.P17-GII.17 strains were found in sporadic cases from France (2013), Italy (2015), the Netherlands and Russia [10,15]. The only study providing a detailed molecular characterisation of a GII.P17-GII.17 norovirus strain detected in Europe comes from Italy. There, the strain was identified in two sporadic cases of acute severe gastroenteritis occurring in February 2015 among young children residing in two distinct Italian regions [15].

GII.P17-GII.17 strains encode a new type of RNA-dependent RNA polymerase and the VP1 capsid protein displays amino acid substitutions in major epitopes. Also, the emerging GII.P17-GII.17 genotype is undergoing a fast diversification which led to two sub-clusters based on RdRp and capsid genes. The two sub-clusters are represented by Kawasaki323 2014, and Kawasaki308 2015 strains, respectively [8]. As demonstrated for GII.4 genotype strains, mutations in VP1 can lead to evasion from host immune system [16].

We report here the detection of GII.P17-GII.17 norovirus isolates highly related to Kawasaki308 strain (99.15% nt sequence similarity in the analysed fragment), identified in Japan in 2015. The sequences described were obtained during an outbreak of acute gastroenteritis in late 2015 in the western part of Romania and were identified in two young children (under five years) and in a young adult (under 30 years) with acute gastroenteritis.

The patients resided in two neighbouring localities. Our molecular analysis based on a DNA fragment of the ORF1-ORF2 (RdRp-VP1) junction (Figure), indicates that the Romanian strains belong to Kawasaki308 2015 sub-cluster (100% bootstrap value). This sub-cluster comprises strains identified in 2014 and 2015 in Hong Kong and in the city of Guangzhou, Guangdong province, China. Gastroenteritis outbreaks caused by this emergent genotype were recorded in the provinces of Jiangsu and Guangdong in China, in 2014 and 2015 [11,12]. Also, the Italian strain [15] and an isolate identified in 2014 in a sporadic case of acute gastroenteritis in a three-year-old child from the United States [17] are closely related to the Romanian isolates.

Conclusion

This documented outbreak, outside Asia, was caused by a norovirus strain belonging to the emergent GII.P17-GII.17 genotype which in the near future might become the dominant genotype in Europe. Due to the limited number of diagnosed cases, we cannot totally exclude the possibility that this gastroenteritis outbreak could have been caused by another pathogen; however, considering the clustering in time and place and rapid onset of symptoms, norovirus was the most probable cause of this outbreak. Therefore, national and European surveillance systems should be prepared for events associated to this emergent strain.

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Conflict of interest

None declared.

Authors' contributions

MN, DGN and EDP collected and analysed epidemiological data. LZ managed the acute diarrhoea syndrome surveillance programme. SD and GO performed sequencing and phylogenetic analysis. SD wrote the paper. GO revised the manuscript.

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Hepatitis B vaccination coverage and risk factors associated with incomplete vaccination of children born to hepatitis B surface antigen-positive mothers, Denmark, 2006 to 2010

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In Denmark, universal screening of pregnant women for hepatitis B has been in place since November 2005, with the first two years as a trial period with enhanced surveillance. It is unknown what the change to universal screening without enhanced surveillance has meant for vaccination coverage among children born to hepatitis B surface antigen (HBsAg)-positive mothers and what risk factors exist for incomplete vaccination. This retrospective cohort study included 699 children of mothers positive for HBsAg. Information on vaccination and risk factors was collected from central registers. In total, 93% (651/699) of the children were vaccinated within 48 hours of birth, with considerable variation between birthplaces. Only 64% (306/475) of the children had received all four vaccinations through their general practitioner (GP) at the age of two years, and 10% (47/475) of the children had received no hepatitis B vaccinations at all. Enhanced surveillance was correlated positively with coverage of birth vaccination but not with coverage at the GP. No or few prenatal examinations were a risk factor for incomplete vaccination at the GP. Maternity wards and GPs are encouraged to revise their vaccination procedures and routines for pregnant women, mothers with chronic HBV infection and their children.

Introduction

Hepatitis B virus (HBV) infection is a worldwide health problem with more than 350 million people estimated to have chronic liver infections caused by HBV [1]. If hepatitis B surface antigen (HBsAg) is detected in the blood for more than six months, the HBV-infection has become chronic [2]. For infants (up to 1 year old) and children (1–10 years) the two primary sources of HBV infection are perinatal transmission from infected mothers and horizontal transmission from infected household contacts [2]. Mother-to-child transmission of HBV can be effectively (95%) prevented by vaccination [1,2]. The risk of becoming chronically infected with HBV is inversely related to the age of the patient at the time of infection [3]. Chronic infection occurs in ca 90% of infected infants, in 30% of infected children younger than five years and in less than 5% of those infected when they are five years or older [2].

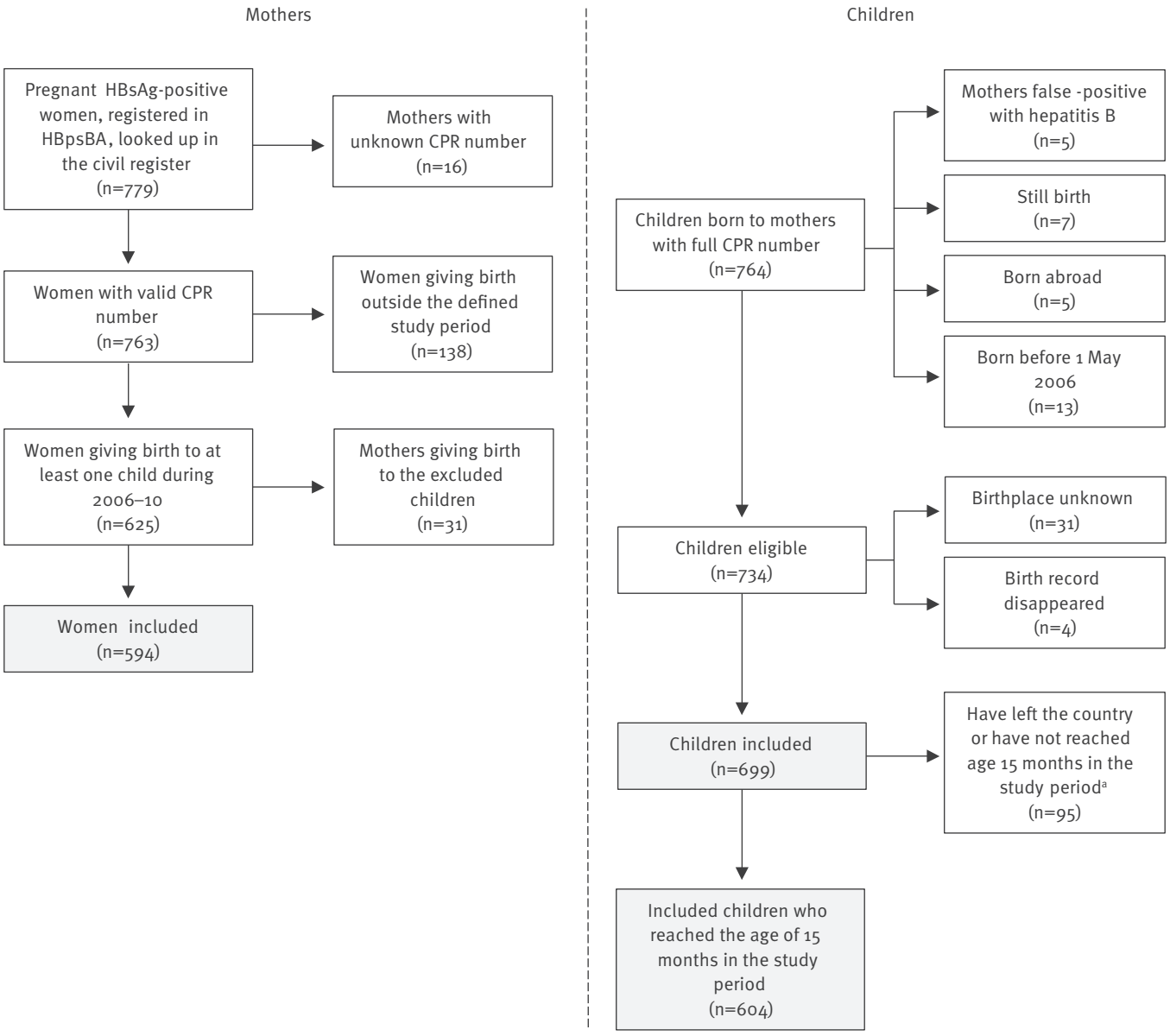
In Denmark, mother-to-child infection is the primary cause (72%) of chronic HBV-infection [4]. Universal screening of pregnant women for hepatitis B has been in place since November 2005 and was made permanent in November 2007 [5,6]. The first two years (1 November 2005 until 31 October 2007) were a trial period with enhanced surveillance. The enhanced surveillance comprised blood banks, maternity wards and general practitioners (GP), for example contacting the maternity wards to secure vaccination of the individual infants and informing the GP about further vaccination of the infants and screening of family members [7,8]. Approximately 180 cases of HBV infection were observed annually between 2006 and 2010, primarily among women from areas highly endemic for hepatitis B [9]. During the trial period, 0.26% of pregnant women were found to be chronically infected with HBV [7].

HBV vaccination is not part of the Danish childhood vaccination programme (CVP) [10,11]. Efforts were instead put into the pregnancy screening programme and into risk group vaccination [12]. This paper presents an evaluation of the pregnancy screening and the subsequent hepatitis B vaccination of the children.

The objective of pregnancy screening is to ensure that all neonates born to HBsAg-positive women are vaccinated against HBV. Furthermore, it is important that GPs refer HBsAg-positive pregnant women to a department for infectious diseases for further information and

Figure 1

Population and samples included in the retrospective cohort study of hepatitis B surface antigen-positive mothers (n = 594) and their children (n = 699 born 2006–2010), Denmark, 2006–2010



CPR: unique identification number in the Danish civil register; HBsAg: Hepatitis B surface antigen; HBpsBA: The national hepatitis B pregnancy-screening database.

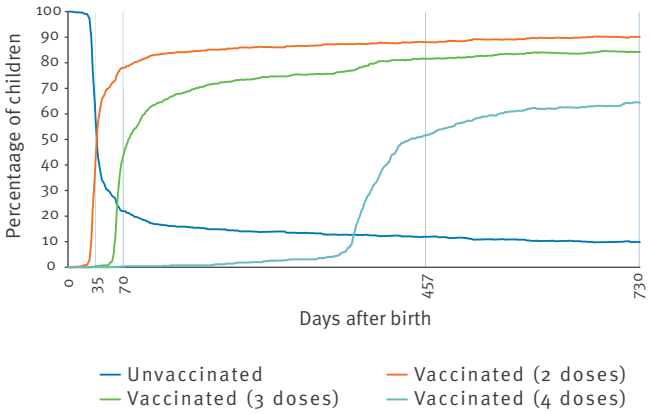
[a] Some children had not reached the age for the fourth vaccination or had left the country.

ongoing care for the newly diagnosed mother including eventually treatment during pregnancy [13]. Only half of the women were referred during the trial period (personal communication: Weiss N, Hvidovre Hospital, Denmark, June 2014, with permission). During the trial period with universal screening, 96% of the children received vaccination at birth [14]. The HBV vaccination coverage of the children born to HBsAg-positive mothers in Denmark during 2008 to 2010 is not known.

According to Danish national guidelines, the vaccination schedule for children born to HBsAg-positive mothers comprises four doses of vaccine, Engerix-B (10 µg): the first dose of vaccine and hepatitis B immunoglobulin (HBIG) at day 0 and three additional doses of vaccine at one, two and 12 months of age [13,15]. All the vaccinations are free of charge [16]. The maternity ward takes care of the first vaccination, including HBIG, and the GPs take care of the rest of the vaccinations.

Figure 2

Cumulative Kaplan–Meier survival curves showing the proportion of children vaccinated for hepatitis B by their general practitioner and unvaccinated children, by days after birth, Denmark, 2006–2010 (n = 699)



The small drop in the beginning of the Unvaccinated curve, is explained by the number of children (n = 48) not having received the birth dose.

Table 1

Country of origin of hepatitis B surface antigen-positive mothers, retrospective cohort study, Denmark, 2006–2010 (n = 594)

Geographical area	Women included in the study	
	n	%
South-east Asia	252	42
The Middle East, northern African countries, including Israel and Turkey	107	18
Sub-Saharan Africa	91	15
Eastern Europe	71	12
Indian subcontinent, including India, Pakistan and Bangladesh	40	7
Denmark	20	3
Greenland	5	< 1
Oceania (Tonga and New Zeeland)	2	< 1
South America (Chile and Brazil)	2	< 1
Western Europe (France (n = 1), Spain (n = 2), Sweden (n = 1))	4	< 1

The aim of this study was to describe the vaccination coverage among children of HBsAg-positive mothers born during the trial period of universal screening with enhanced surveillance (2006–2007) or born in the following three years with universal screening without enhanced surveillance (2008–2010), to identify risk factors for incomplete vaccination and to discuss possibilities for additional prevention. This article is a proof of concept.

Methods

The study was a retrospective cohort study of pregnant HBsAg-positive women and their live-born children in Denmark from 2006 to 2010. The first two years of the universal screening constituted a trial period with enhanced surveillance.

All Danish residents are registered with a unique identification number (CPR number) [17]. From the national hepatitis B pregnancy screening database (HBpsBA), we know that around 2% of the pregnant women notified with hepatitis B do not have a CPR number. Since they are not listed in the civil register, they were not a part of this study.

Data sources and registers

The national hepatitis B pregnancy-screening database

Statens Serum Institute cooperates with the blood banks on monitoring the pregnancy screening in Denmark [15]. The database includes data on all HBsAg-positive women screened since 2005. From this database we extracted all women screened in the defined study period. Data on name, CPR number and date of screening were pulled from the database.

The civil register

This register was established in 1968. Danish residents are registered with a CPR number [17]. From this register, we obtained the CPR numbers of children born in Denmark to mothers known to be infected with HBV and still residing in Denmark. Further, we extracted data on country of origin and immigration/emigration data of the mothers of these women.

The service code register (in Danish: Sygesikringsregisteret)

This is a database containing all services given by primary healthcare practitioners. Each service has a code and each code is registered by CPR number. The database is updated monthly. We extracted service codes for HBV vaccinations of children born to HBsAg-positive mothers (code: 8314–8316), service codes for the prenatal visits (code: 8110–8130) and health examinations of the mothers eight weeks after giving birth (code: 8140).

The national birth register

This register was established in 1973. From this register we identified the hospitals where the HBsAg-positive mothers gave birth.

The maternity wards in Denmark

Data on HBV vaccination and HBIG at birth are registered at the hospitals. All maternity wards were contacted. Data collected were: time of birth, HBV vaccine given (yes/no), HBIG given (yes/no) and if yes for any of these, date and time.

The general practitioners

If data on vaccination were missing in the service code register, the GPs were contacted by phone.

Table 2

Children vaccinated against hepatitis B at birth and at the general practitioner's, by birth year, Denmark, 2006–2010 (n = 699)

Birth year	Live births	Birth vaccine				Vaccinations at the general practitioner's						All vaccinations	
		Vaccinated within 24 hours[a]		Vaccinated within 48 hours[b]		Second dose received within 5 weeks		Third dose received within 10 weeks		Fourth dose received within 15 months		All four doses received within 15 months	
		n	%	n	%	n	%	n	%	n	%	n	%
2006	100	89/100	89	93/100	93	47/100	47	48/100	48	59/99[c]	60	57/99[c]	58
2007	155	146/155	94	153/155	99	78/155	50	73/155	47	82/152[c]	54	80/152[c]	53
2008	161	133/161	83	139/161	86	66/161	41	66/161	41	78/160[c]	49	74/160[c]	46
2009	158	148/158	94	149/158	94	55/158	35	55/158	35	78/156[d]	50	78/156[d]	50
2010	125	112/125	90	117/125	94	59/125	48	62/125	50	15/37[d]	41	15/37[d]	41
Total	**699**	**628/699**	**90**	**651/699**	**93**	**305/699**	**44**	**304/699**	**43**	**312/604[d]**	**52**	**304/604[d]**	**50**

Vaccination schedule in Denmark: At birth (within 48 hours), and at age 1, 2 and 12 months.

The table is divided in columns of birth vaccines and columns of vaccinations at the general practitioner. Within these groups the numbers are summed up. For instance, when looking at the third dose received, these numbers include both second and third dose received, when the child is 10 weeks-old and so forth. The last column shows those children who have received all four doses of vaccine.

[a] Both hepatitis B immunoglobulin (HBIG) and hepatitis B vaccine received, except in 10 children who had only received the vaccine.

[b] Both HBIG and hepatitis B vaccine received, except in 11 children who had only received the vaccine. Four children were vaccinated at the delivery site, but after 48 hours, and are regarded as unvaccinated (and not included in the column).

[c] Some children had left the country.

[d] Some children had not reached the age for the fourth vaccination or had left the country.

Study population

We included all HBsAg-positive mothers registered in HBpsBA from 1 November 2005 until 10 January 2011 and the children they gave birth to from 1 May 2006 onwards (Figure 1).

Definition of 'vaccinated in time'

Scheduled vaccination of children born to HBsAg-positive mothers according to the national vaccination schedule is immediately/within 48 hours after birth, and at the age of one, two and 12 months. We used the following limits for timely vaccination:

- First vaccination: immediate vaccination after birth/ within 48 hours
- Second vaccination: 5 weeks after birth (nationally scheduled at 4 weeks)
- Third vaccination: 10 weeks after birth (nationally scheduled at 8 weeks)
- Fourth vaccination: 15 months after birth (nationally scheduled at 12 months)

Risk factors for missing vaccination

We analysed risk factors for incomplete vaccination. The following risk factors were included: the mothers' country of origin, year of delivery, number of pregnancy examinations, mother's age when giving birth and length of time in Denmark before giving birth. We were able to extract all risk factors from the registers.

Statistical analyses

Kaplan–Meier survival curves were used to estimate the probability for vaccination and how many days after birth the children were vaccinated. The regression model used in this study was logistic regression estimating odds ratios (OR) of included risk factors. The risk factor analyses had three steps: (i) univariate analysis for each risk factor separately, (ii) multivariate analysis with all risk factors included, and (iii) multivariate analysis, final model, with backward elimination with a threshold at 10%. When discussing changes in risk we used a binary regression (Poisson regression without exposure) to estimate risk ratios (RR). We used a significance level of 5%. All data were analysed in STATA version 10.0.

Results

After exclusion of mothers with unknown CPR number, mothers who had given birth outside the defined study period, mothers who had given birth to excluded children and children having left the country, the final cohort comprised 699 children and 594 mothers (Figure 1).

Characteristics of the mothers

The median age at delivery was 31 years (range: 17–44 years). Of the 594 mothers, 267 (45%) had previously given birth in Denmark. Thirty-five (6%) of the mothers were adopted as children from high-endemic countries for hepatitis B, and 252 mothers (42%) originated from south-east Asia, a high-endemic area (Table 1).

Vaccination of the children

Of the 699 children, 651 (93%) were vaccinated within 48 hours after birth, 628 (90%) within 24 hours. A total of 305 (44%) of the 699 children had received the second dose of vaccine from their GP at five weeks of age and 304 (43%) had received the third dose 10 weeks after birth. Of the 604 children who had reached the age of 15 months during the study period, 312 (52%)

Table 3

Factors associated with not receiving hepatitis B vaccination within 48 hours of birth, Denmark, 2006–2010 (n = 699)

Factor	Level	Liveborn children n = 699		Newborns not receiving vaccination[a] n = 48		Single factor analysis		Multivariate analysis (all factors)		Multivariate analysis (final model)[d]	
		n	%	n	%	OR (95%CI)	p [e]	OR (95% CI)	p [e]	OR (95% CI)	p [e]
Country of origin[b]	Danish Not Danish	23 676	3 97	8 40	17 83	1 (ref) 0.12 (0.05–0.29)	<0.01	1 (ref) 0.07(0.02–0.21)	<0.01	1 (ref) 0.11 (0.041–0.27)	<0.01
Year of delivery	2006 2007 2008 2009 2010	100 155 161 158 125	14 22 23 23 18	7 2 22 9 8	15 4 46 19 17	5.76 (1.17–28.34) 1 (ref) 12.11 (2.79–52.49) 4.62 (0.98–21.77) 5.23 (1.09–25.12)	<0.01	6.67 (1.21–36.90) 1 (ref) 15.80 (3.36–74.25) 5.30 (1.05–26.71) 5.66 (1.11–28.85)	<0.01	5.85 (1.10–31.04) 1 (ref) 13.06 (2.85–59.90) 5.02 (1.02–24.80) 5.51 (1.10–27.67)	<0.01
Pregnancy examinations	None 1 2 3	17 97 125 460	2 14 18 66	1 8 8 31	2 17 17 65	1 (ref) 1.44 (0.17–12.32) 1.09 (0.13–9.35) 1.16 (0.15–9.02)	0.95	1 (ref) 1.80 (0.28–11.47) 1.21 (0.19–7.73) 1.21 (0.20–7.18)	0.80	NA	NA
Age group (mothers age when giving birth)	17–24 years 25–29 years 30–34 years 35–44 years	66 217 255 161	9 31 36 23	4 11 22 11	8 23 46 23	1 (ref) 0.83 (0.25–2.69) 1.46 (0.47–4.41) 1.14 (0.35–3.71)	0.50	1 (ref) 1.26 (0.37–4.29) 2.84 (0.88–9.20) 1.62 (0.49–5.40)	0.12	NA	NA
Total time in Denmark before giving birth[c]	<1 year 1–5 years >5 years	n = 676 45 113 518	7 17 77	n = 40 5 5 30	13 13 75	1 (ref) 0.37 (0.10–1.35) 0.49 (0.18–1.34)	0.28	1 (ref) 0.27 (0.08–0.98) 0.35 (0.12–1.00)	0.11[e]	NA	NA

NA: not applicable; OR: odds ratio CI: confidence interval; ref: reference value.

[a] Vaccination means both hepatitis B immunoglobulin and vaccine received within 48 hours, except in 10 children who only received the vaccine.

[b] Country of origin is divided into only two levels, Danish and not Danish, since the single variable analysis showed no difference (p = 0.98) between any other ethnic groups compared with the Danish group. The other ethnic groups came from: Greenland, Indian subcontinent (including India, Pakistan and Bangladesh), the Middle East and Africa north of Sahara (including Turkey and Israel), Oceania, Africa south of Sahara, South America, south-east Asia, western and eastern Europe.

[c] Total time in Denmark is the accumulated time in the country for one person. If a person was travelling in and out of the country, the time not in Denmark was subtracted from the total time. Only mothers with non-Danish ethnicity were included in this variable. Only one pregnant woman arrived in Denmark less than six months before delivery.

[d] Final model: stepwise backward elimination at 10% level.

[e] The p values for total time in Denmark before giving birth refer to the joint effect/the overall significance of the factor, although the individual levels in the factor were significant.

had received three doses of vaccine at the GP. Half of the 604 children (n = 304) had received all four doses (one at birth and three doses at the GP's) (Table 2). At the time of the study, 95 of the total 699 children had not reached the age for the fourth dose of vaccine or had left the country (Figure 1) and could therefore not be followed up at age 15 months.

HBV vaccinations were under-reported to the service code register. For 325 of the 699 children (46%), the second dose of HBV vaccine (the first given by the GP) had not been recorded in the register by the GP. However, contact to the GPs revealed that of these 325 children, 251 (77%) had in fact received the second dose. For the third and fourth dose, the corresponding proportions were 64% (208/327) and 36% (166/457), respectively.

Experience from the collection of data in several cases showed that the reason for a disrupted vaccination schedule was that the mother changed GP.

Table 4

Factors associated with an incomplete course of hepatitis B vaccine (less than four doses) at 15 months of age, Denmark, 2006–2010 (n = 604)

Factor	Level	Liveborn children n = 604		Children with incomplete vaccination n = 300		Single factor analysis		Multivariate analysis (all factors)		Multivariate analysis (final model)[d]	
		n	%	n	%	OR (95%CI)	p	OR (95% CI)	p	OR (95% CI)	p
Received vaccination at birth[a]	Yes No	565 39	94 6	261 39	87 13	[a] 1 (ref)	<0.01*	NA	NA	NA	NA
Country of origin[b]	Danish Not Danish	20 584	3 97	12 288	4 96	1 (ref) 0.64 (0.26–1.61)	0.35	1 (ref) 1.08 (0.34–3.36)	0.90	NA	NA
Year of delivery	2006 2007 2008 2009 2010	99 152 160 156 37	16 25 26 26 6	42 72 86 78 22	14 24 29 26 7	0.82 (0.49–1.36) 1 (ref) 1.29 (0.82–2.02) 1.11 (0.71–1.74) 1.63 (0.79–3.38)	0.30	0.68 (0.39–1.17) 1 (ref) 1.03 (0.64–1.65) 1.02 (0.64–1.64) 1.58 (0.71–3.53)	0.34	NA	NA
Pregnancy examination	None 1 2 3	15 84 104 401	2 14 17 66	10 52 55 183	3 17 18 61	1 (ref) 0.81 (0.25–2.60) 0.56 (0.18–1.76) 0.42 (0.14–1.25)	0.02	1 (ref) 0.73 (0.20–2.63) 0.53 (0.15–1.84) 0.40 (0.12–1.36)	0.07	1 (ref) 0.45 (0.24–0.86) 0.56 (0.29–1.05) 0.76 (0.39–1.48)	0.03
Post-pregnancy examination	Yes No	364 240	60 40	167 133	56 44	0.68 (0.71–0.97) 1 (ref)	0.02	0.73 (0.51–1.04) 1 (ref)	0.08	NA	NA
Age group (mothers' age when giving birth)	17–24 years 25–29 years 30–34 years 35–44 years	58 196 215 135	10 32 36 22	36 84 106 74	12 28 35 25	1 (ref) 0.45 (0.25–0.84) 0.59 (0.33–1.08) 0.74 (0.39–1.39)	0.04	1 (ref) 0.46 (0.23–0.89) 0.55 (0.29–1.06) 0.76 (0.39–1.51)	0.05	1 (ref) 0.75 (0.21–2.62) 0.54 (0.16–1.85) 0.39 (0.12–1.29)	0.03
Total time in Denmark before giving birth[c]	<1 year 1–5 years >5 years	n = 584 37 91 456	6 16 78	n = 288 20 48 220	7 17 76	1 (ref) 0.95 (0.44–2.04) 0.79 (0.40–1.55)	0.62	1 (ref) 1.44 (0.60–3.43) 1.18 (0.54–2.56)	0.64	NA	NA

NA: not applicable; OR: odds ratio; CI: confidence interval; ref: reference value.

Only mothers who had not left Denmark at the 15-months examination are included in the Table.

[a] Vaccination means both hepatitis B immunoglobulin and vaccine received at the birth place any time before 48 h, except in 11 children who only received the vaccine. Because all live born children with no vaccination at birth (n = 39) were exactly the same as those with incomplete vaccination at 15 months of age, the OR could not be estimated for this group. Therefore, this group was excluded from the multivariate analysis; in the univariate analysis, a Fisher's exact test could calculate the significance of this factor.

[b] Country of origin is divided into only two levels, Danish and not Danish, since the single variable analysis showed no difference (p = 0.98) between any other ethnic groups compared with the Danish group. The other ethnic groups came from: Greenland, Indian subcontinent (Including India, Pakistan and Bangladesh), the Middle East and Africa north of the Sahara (including Turkey and Israel), Oceania, Africa south of the Sahara, South America, south-east Asia, western and eastern Europe.

[c] Total time in Denmark is the accumulated time in the country for one person. If a person was travelling in and out of the country, the time not in Denmark was subtracted from the total time. Only mothers with non-Danish ethnicity were included in this variable. Only one pregnant woman arrived in Denmark less than six months before delivery.

[d] Final model: stepwise backward elimination at 10% level.

*Statistically significant in Fischer's exact test.

Box

Lessons learned

- Vaccination procedures and routines at the sites of delivery with optimal organisation (best practice) should be a model to implement at all sites.
- The sites of delivery should be attentive to mothers of foreign origin but not forget mothers of Danish origin with chronic HBV infection as well as mothers who were themselves adopted.
- It is important that GPs are informed about vaccinations initiated at the hospital and know the plan for subsequent treatment.
- The GP should pay particular attention to ensure that pregnant women with chronic HBV infection attend all prenatal examinations.
- If it becomes known that a child has not received the vaccination series as recommended, the child should be called in for vaccination.
- If the child changes GP, it is important to communicate the HBV vaccination status to the new GP.
- It is important that the GP employ the special provider number for the vaccine, which forms the basis of any assessment of the vaccination coverage nationally.

In addition to how many children received the recommended number of doses of vaccine, we also analysed the timeliness of the vaccines given. Figure 2 shows the total number of unvaccinated children and when the children obtained the individual vaccines at the GP's. At the age of 10 weeks, 78% (545/699) of the children had received the second dose. At the age of two years, 64% (306/475) of the children had received all four vaccinations. At the age of 15 months (457 days), 12% (72/604) of the children had not received any HBV vaccinations at all; this figure had dropped to 10% (47/475) by the age of two years (Figure 2).

Risk factors associated with not receiving hepatitis B vaccination within 48 hours after birth

Year of delivery was significantly correlated with risk of not being vaccinated: Giving birth in 2008, just after the enhanced surveillance was terminated, had a 12 times higher risk (relative risk (RR) = 11.70; 95% confidence interval (CI): 2.80–48.80) of not being vaccinated than giving birth in 2007 (Table 3; odds ratio (OR) = 13.06; 95% CI: 2.85–59.90). This increased risk declined in the following years, but was still five times higher (RR = 4.84; 95% CI: 1.14–20.53) in 2009 and 2010 together than in 2007.

For children of HBsAg-positive non-Danish mothers, the risk of missing vaccination at the time of birth was 87% lower (RR = 0.13; 95% CI: 0.063–0.25) than for children of Danish HBsAg-positive mothers (Table 3).

Neither age of the mother when giving birth nor total time in Denmark before giving birth were significantly correlated with the risk of not being vaccinated. Likewise, the number of pregnancy examinations had no statistically significant influence on the risk of not being vaccinated at birth. Finally, it made no difference in which region of Denmark the birth had taken place, nor was there any difference in the risk of missed vaccination at birth between urban and rural hospitals.

Ten sites of delivery had vaccinated all children, while the remaining 19 sites each counted between one and seven cases of lacking vaccination at birth.

The multivariate models fit well. For the all-factors and final-model analysis in Table 3, the R2 coefficients were, respectively, 0.1349 and 0.1065.

Risk factors associated with less than four hepatitis B vaccinations by the age of 15 months

Newborns who had not received HBV vaccine at birth had a significantly higher risk of incomplete vaccination at 15 months (Table 4). The number of prenatal examinations was also significantly correlated with the completeness of vaccination, more prenatal examinations being associated with a lower risk of incomplete vaccination. Finally, the mothers' age was significantly correlated with the risk of incomplete vaccination at 15 months: the youngest (17–24 years) and the oldest (35–44 years) age groups of mothers had the highest risk of incomplete vaccination of their children.

Neither the mothers' country of origin nor their total time in Denmark were significantly correlated with the risk of incomplete vaccination. In contrast to vaccination at birth, the year of delivery had no effect on the risk of incomplete vaccination at the GP's (Table 4).

In the multivariate analysis, there was no effect of the enhanced surveillance in 2007 (Table 4). We even observed a slight, but not significant, increasing trend (p = 0.08) over the years in the risk of incomplete vaccination at 15 months of age. Whether or not the mother had had a post-pregnancy examination did not significantly influence the risk of incomplete vaccination at 15 months, although there was a tendency towards a higher risk for those not having received this examination, the RR being 0.87 (95% CI: 0.74–1.02) (p = 0.08).

The multivariate models fit well. For the all-factors and final-model analysis in Table 4, the R2 coefficients were respectively 0.0347 and 0.0237.

Discussion

This retrospective cohort study comprised a total of 699 children born to HBsAg-positive mothers. The participation in the study was 95.2% (four birth records had disappeared or birth site was unknown for 31 children) (Figure 1).

The overall vaccination coverage 48 hours after birth was 93% (651/699) (Table 2). Missing vaccination is considered an adverse event and is subject to internal auditing at the hospital. It is discouraging to find that such a high proportion of the children were not vaccinated according to schedule. Furthermore, 10% (47/475) of the children had not received a single vaccine at the age of two, a dire sign of missed opportunities (Figure 2).

Considerable under-reporting of hepatitis B vaccinations by the GPs to the service code register was revealed. Had we not contacted the GPs, the results would have been misleading.

Compared with other European countries, the percentage of vaccination at birth was lower in Denmark. In a study in London in 2006, 97% of the children received their birth vaccination and 49% of the children had received four vaccines by the age of 15 months [18]. In a Swiss study from 2010, 99% of newborns received their vaccination within 24 hours [19]. In the same study, the vaccination series was completed in 83% of the children in that they had received at least two doses besides the birth dose. In a study in Amsterdam in 2001, 96% of all newborns received HBIG within 24 hours and 91% of the children received their third vaccination dose on time (within seven months after the second vaccination) [20]. We expected the Danish birth vaccination percentage to be higher because the standard procedures for this group of women were supposed to be incorporated as routine after the two-year trial period with enhanced surveillance during which the midwives were obliged to fill in questionnaires regarding all children born to HBsAg-positive mothers. In the Netherlands, the Municipal Health Service played a major role in the organisation and follow-up of the children's vaccination status, in contrast to the Danish system.

Variation in birth vaccination coverage was observed between the time periods analysed. In the period with enhanced surveillance (1 November 2005 to 31 October 2007) [8], vaccination coverage at the sites of birth was considerably higher than after termination of the monitoring efforts. In 2007, when the routines had become standard procedure at the sites of birth, only 1% did not receive vaccination at birth (Table 2). Since 2009, the vaccination procedures and coverage have remained at the level they had without enhanced surveillance (94% vaccinated). No effect of the monitoring year was observed for vaccinations at the GPs. We did not find any other studies describing the difference between periods with and without enhanced surveillance.

The salient point is to set a limit for 'vaccinated in time'. To calculate the vaccination coverage at exactly the date scheduled would give an underestimation of the coverage. Still, the date should reflect whether or not the child has been vaccinated according to the national programme and protected against HBV infection. The time limit for each of the vaccinations (doses two to four) in this study was set based upon the Summary of Product Characteristics (SPC) for Engerix-B [21], minimum/maximum intervals [22] and practicalities around the examination schedules for mother and child. According to the Danish national guidelines, the second dose of vaccine can be postponed until the child is five weeks old [15]. This is also the time for the first (of six) routine scheduled childhood examinations at the GP's. The London study from 2006 looking at risk factors used the limit of 15 months for the fourth dose of vaccine [18]. The same limit was used in our study, an extension of 25% compared with the national schedule that foresees this vaccination at 12 months. We also increased the two other vaccination times with 25% for our study, to respectively five and 10 weeks.

Other studies have focused on setting the limit for 'vaccinated in time' [2,19,23-25]. A study from Thailand found a 3.74 times (95% CI: 0.97–14.39) increased risk of the child being chronically infected with HBV if the interval between the first and second vaccine dose was more than 10 weeks [24]. It has been described that vaccination and HBIG at birth, at one and at six months, compared with at birth, at two and at six months, lead to the same protection from acute and chronic HBV infection in children born to HBsAg- and HBeAg-positive mothers [2]. A study form Switzerland did not find any evidence of age at vaccination (one instead of two months-old) having an influence on prevention of HBV infection [19].

The likelihood of having completed vaccinations at the age of 15 months (but not vaccinations at birth) was positively associated with the mother's number of prenatal examinations, as it was in a study in London from 2006 [18]. Although not significant (Table 3), there seemed to be a slightly lesser risk of not being vaccinated at all, if the mother had no prenatal examinations compared with having one, two or three examinations (Table 3 and 4). This is probably because women with no prenatal examinations are often seen in the maternity ward at birth without being registered before. Thus they have their blood sample taken just before giving birth and/or the newborns are vaccinated without knowledge of the mothers' hepatitis B status.

Ethnicity and total time in the country before giving birth were without significance both in our study and the London study [18]. We were not able to assess the mothers' level of integration into Danish society, nor their command of Danish. In the London study, more than basic command of English was correlated with completed vaccination status. Very few mothers in the study were of Danish origin. The children of these mothers had a significantly higher risk of not being vaccinated than children of mothers from high-prevalence countries. The low prevalence in Denmark could mean that attention was not paid to the small group of HBsAg-positive Danish women. In our study, 6% (35/594) of the mothers were themselves adopted. The

hepatitis B status of the adoptees was often unknown by the GP [7]. In Denmark, it is recommended to test adopted children of non-Danish origin for HBV upon arrival in the country [26]. An American study from 2008 found a 4% prevalence for HBV in internationally adopted children [27], which is also high compared to an estimated prevalence of chronic hepatitis B of 0.2–0.3% in the general population in Denmark [14].

Denmark is among the six European countries that have not yet adopted the World Health Organization (WHO) recommendation for inclusion of hepatitis B vaccine in all national vaccination programmes [10-12,28]. These six countries (Denmark, Finland, Iceland, Norway, Sweden and the UK) have adopted risk group-targeted hepatitis B vaccination only [29]. In Denmark, even with hepatitis B vaccination included in the CVP, it will still be necessary to maintain the general screening of pregnant women for HBV, with subsequent vaccination of their newborns against HBV. Including hepatitis B vaccination in the CVP would not save the children of hepatitis B-infected mothers from being infected at birth because the first childhood vaccination is not given before the age of three months.

Our study has some limitations. It has not been possible to investigate other potentially relevant risk factors for incomplete vaccination that could help target preventive measures, such as socioeconomic status, written information about hepatitis B given to the mother and whether the hospital record contains information from the GP.

Since no new national initiatives were implemented during the study period, we have no reason to believe that the coverage has improved or the risk factors have changed after the study period. Since January 2014, parts of the enhanced surveillance of the screening have been revived and since 15 May 2014, a national reminder system has been implemented [30]. The reminder concerning the hepatitis B vaccination of children born to mothers infected with HBV will be sent to the GP as soon as possible after birth. Following the results from this study, it is now recommended that GPs test the infants for protective antibody levels after completed vaccination schedule.

Conclusion

In Denmark, timely and complete HBV vaccination of children born to HBsAg-positive mothers need to be optimised. Sites of birth and GPs are encouraged to revise their vaccination procedures and routines for the group of all pregnant women, mothers with chronic HBV infection and their children. Future studies will show if the resumed national monitoring beginning in 2014 will lead to increased vaccination coverage.

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Conflict of interest

None declared.

Authors' contributions

Asja Kunoee: data collection, data management and analysis, writing the manuscript.

Jens Nielsen: data management and advanced data analysis, commenting the manuscript.

Susan Cowan: data analysis, manuscript co-writer, Danish hepatitis B pregnancy screening background knowledge.

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Research article

Vaccine effectiveness in preventing laboratory-confirmed influenza in primary care patients in a season of co-circulation of influenza A(H1N1)pdm09, B and drifted A(H3N2), I-MOVE Multicentre Case–Control Study, Europe 2014/15

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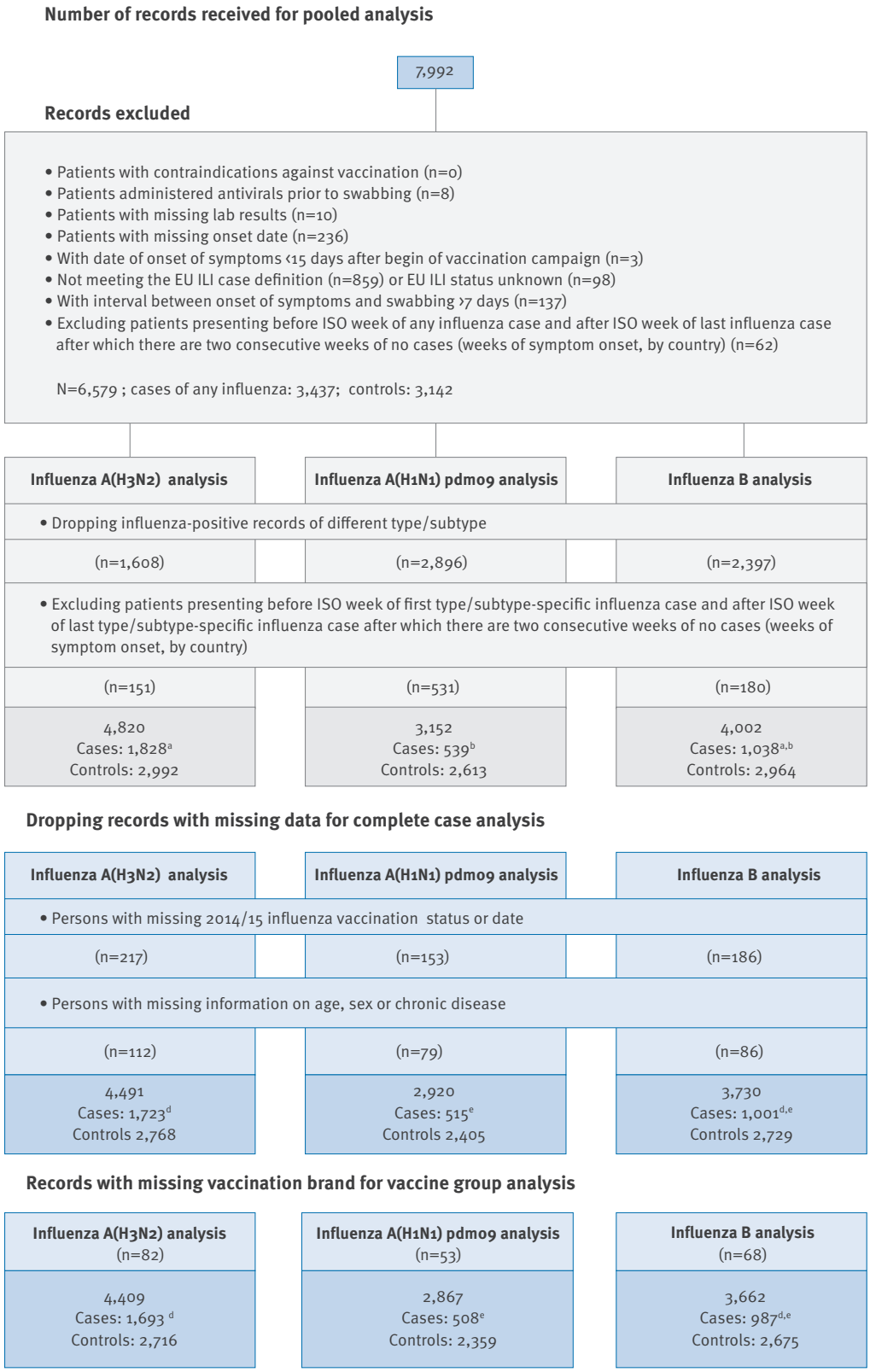
Influenza A(H3N2), A(H1N1)pdm09 and B viruses co-circulated in Europe in 2014/15. We undertook a multicentre case–control study in eight European countries to measure 2014/15 influenza vaccine effectiveness (VE) against medically-attended influenza-like illness (ILI) laboratory-confirmed as influenza. General practitioners swabbed all or a systematic sample of ILI patients. We compared the odds of vaccination of ILI influenza positive patients to negative patients. We calculated adjusted VE by influenza type/subtype, and age group. Among 6,579 ILI patients included, 1,828 were A(H3N2), 539 A(H1N1)pdm09 and 1,038 B. VE against A(H3N2) was 14.4% (95% confidence interval (CI): -6.3 to 31.0) overall, 20.7% (95%CI: -22.3 to 48.5), 10.9% (95%CI -30.8 to 39.3) and 15.8% (95% CI: -20.2 to 41.0) among those aged 0–14, 15–59 and ≥60 years, respectively. VE against A(H1N1) pdm09 was 54.2% (95%CI: 31.2 to 69.6) overall, 73.1% (95%CI: 39.6 to 88.1), 59.7% (95%CI: 10.9 to 81.8), and 22.4% (95%CI: -44.4 to 58.4) among those aged 0–14, 15–59 and ≥60 years respectively. VE against B was 48.0% (95%CI: 28.9 to 61.9) overall, 62.1% (95%CI: 14.9 to 83.1), 41.4% (95%CI: 6.2 to 63.4) and 50.4% (95%CI: 14.6 to 71.2) among those aged 0–14, 15–59 and ≥60 years respectively. VE against A(H1N1)pdm09 and B was moderate. The low VE against A(H3N2) is consistent with the reported mismatch between circulating and vaccine strains.

Introduction

In February 2014 each year, the World Health Organization (WHO) provides recommendations for the composition of the northern hemisphere vaccines, based on information from the WHO Global Influenza Surveillance and Response System. In 2014, the WHO vaccine strain selection committee recommended that the 2014/15 northern hemisphere influenza vaccine should include the same components as in 2013/14: an A/California/7/2009 (H1N1)pdm09-like

Figure 1

Flowchart of data exclusion for pooled analysis, I-MOVE multicentre case–control study, Europe, influenza season 2014/15 (week 41/2014-week 19/2015)



EU: European Union; ILI: influenza-like illness; I-MOVE: Influenza Monitoring Vaccine Effectiveness in Europe; ISO: International Organization for Standardization.

[a] Includes 15 influenza B + A(H3N2) co-infections.

[b] Includes 8 influenza B + A(H1N1)pdm09 co-infections.

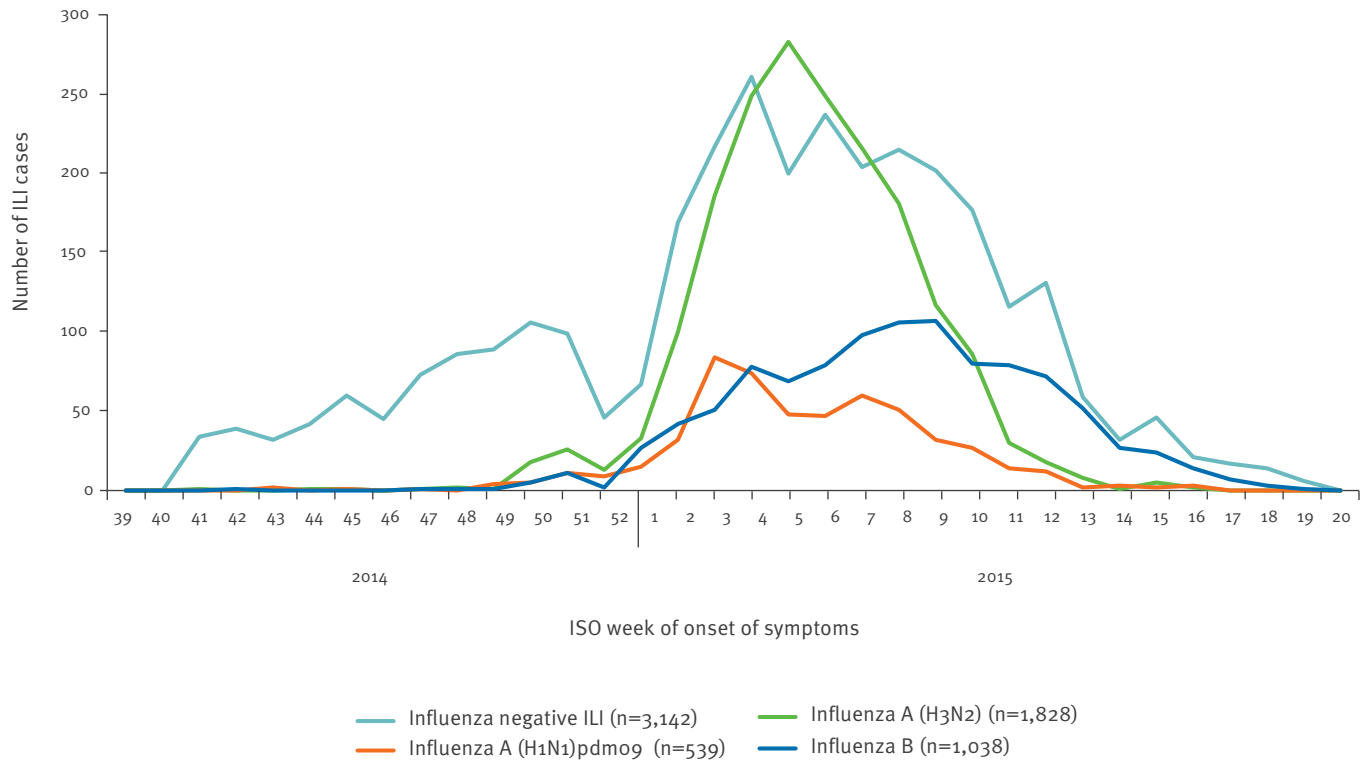
[c] Includes 3 influenza B + A(H3N2)pdm09, and 7 A(H1N1)pdm09 + A(H3N2) co-infections.

[d] Includes 14 influenza B + A(H3N2)pdm09 co-infections.

[e] Includes 7 influenza B + A(H1N1)pdm09 co-infections.

Figure 2

Number of influenza-like illness reports by case status and week of symptom onset, all influenza, target groups for vaccination, I-MOVE multicentre case–control study, Europe, influenza season 2014/15 (week 41/2014-week 19/2015) (n=6,524[a])



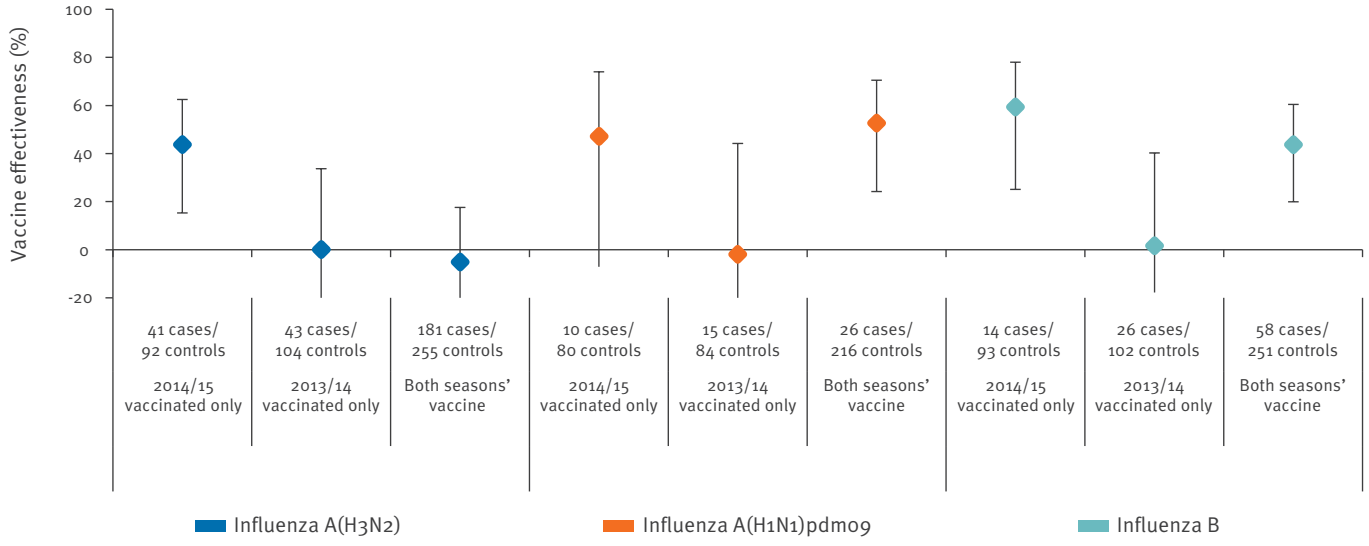
ILI: influenza-like illness; I-MOVE: Influenza Monitoring Vaccine Effectiveness in Europe, ISO: International Organization for Standardization.

[a] This includes 15 influenza B + A(H3N2) co-infections and eight influenza B + A(H1N1)pdm09 co-infections. Note that numbers of cases come from influenza type/subtype specific databases. Some cases are excluded due to their restriction criteria. Any influenza A non-typed cases are dropped from analysis.

The proportion vaccinated with the 2014/15 influenza vaccine was 13.2% among controls, 13.0% among A(H3N2) cases, 6.9% among A(H1N1) pdm09 cases and 7.4% among B cases (Table 2).

Figure 3

Pooled crude and adjusted seasonal vaccine effectiveness against laboratory confirmed influenza by influenza type/subtype, and by season of vaccination, I-MOVE multicentre case–control study, Europe, influenza season 2014/15 (week 41/2014-week 19/2015)



I-MOVE: Influenza Monitoring Vaccine Effectiveness in Europe.

virus, an A/Texas/50/2012 (H3N2)-like virus, and a B/Massachusetts/2/2012-like virus [1].

In September 2014, the WHO reported the emergence of two new influenza virus genetic clades for A(H3N2), clade 3C.2a and 3C.3a [1]. These clades had first circulated in Europe during the 2013/14 influenza season [2].

In December 2014, the United States (US) Centers for Disease Control and Prevention (CDC) issued a Health Alert reporting that 52% of the A(H3N2) viruses circulating were antigenically different from the A(H3N2) component of the northern hemisphere 2014/15 influenza vaccine. CDC recommended the use of antiviral medications where indicated for the treatment and prevention of influenza, as an adjunct to vaccination [3]. Concordant with the reports of the drifted A(H3N2) viruses, in January 2015, the US, Canada and the United Kingdom (UK) reported low influenza vaccine effectiveness (VE) against A(H3N2) [4-6]. Canadian results suggested that VE against influenza A(H3N2) among individuals who had been vaccinated in both 2013/14 and 2014/15 seasons was lower than among those who were only vaccinated in 2014/15 [5].

In Europe, the influenza season started later than in the US and Canada. Increased influenza activity in Europe was first reported in early January 2015, with a predominance of A(H3N2) but with influenza A(H1N1) pdm09 and B circulating as well [7].

For this seventh season of the Influenza Monitoring Vaccine Effectiveness in Europe (I-MOVE) multicentre case–control study we aimed to measure the 2014/15 effectiveness of the seasonal influenza vaccine against the three co-circulating viruses by age group and by vaccine type. In addition, due to the potential implications for vaccination policy we explored the effect of previous vaccinations on the current season VE.

Methods

Eight study sites (Germany, Hungary, Ireland, Italy, Poland, Portugal, Romania and Spain) participated in the test-negative 2014/15 multicentre case–control study. The methods have been described previously [7-9] and are based on the European Centre for Disease Prevention and Control (ECDC) generic case–control study protocol [10].Briefly, participating general practitioners (GPs) interviewed and collected naso-pharyngeal specimens from all (seven study sites) or a systematic sample (in Germany) of patients consulting for influenza- like illness (ILI) aged 60 (Germany, Poland, and three regions in Spain) or 65 years old (Hungary, Ireland, Italy, Portugal, Romania and three regions in Spain) and older and from a systematic sample of ILI patients in the other age groups. In Hungary, only patients aged 18 years or over were eligible for inclusion in the study. GPs collected clinical and epidemiological information as previously described [8]. We included patients in the study who presented to the GPs more than 14 days after the start of the national vaccination campaigns and who met the European Union (EU) ILI case definition [11], were swabbed within seven days of symptom onset, and who had not received antivirals before swabbing.

Cases were ILI patients who were swabbed and tested positive for influenza virus using real-time reverse-transcription PCR (RT-PCR). Controls were ILI patients who tested negative for any influenza virus using RT-PCR. Cases and controls were not included in the influenza type/subtype-specific analyses if fewer than five type/subtype-specific cases were reported by study site. Influenza A cases of unknown subtype were excluded from the analysis.

For each study site and for each influenza type/subtype, the study period started on the week of onset of the first influenza case recruited and ended on the week of onset of the last influenza case after which there were at least two consecutive weeks with no further influenza positive cases.

We defined a patient as vaccinated if they had received minimum one dose of 2014/15 influenza vaccine at least 15 days before ILI symptom onset. We considered all other patients unvaccinated. GPs ascertained vaccination based on vaccination records or patient's self-report.

For each study site, we compared the odds of vaccination in cases and controls calculating the odd ratio (OR). We conducted a complete case analysis excluding patients with missing values for any of the variables in the model measuring adjusted VE. We carried out a one-stage model with study site as a fixed effect. We used Cochran's Q-test and the I^2 index to test the heterogeneity between study sites [12].

We used a logistic regression model to calculate VE including potential confounding factors: age (modelled as a restricted cubic spline with four knots or age group as a categorical variable depending on the analysis), sex, presence of at least one underlying chronic condition (including pregnancy and obesity where available) and date of symptom onset (modelled as a restricted cubic spline with four knots where sample size allowed).

To study the effect of 2013/14 vaccination on the 2014/15 VE, we conducted a stratified analysis using four categories: individuals unvaccinated in both seasons (reference category), vaccinated in 2013/14 only, vaccinated in 2014/15 only, and those vaccinated in both seasons.

We measured VE by age group (0–14, 15–59 and ≥60 years) and by type of vaccine (adjuvanted, egg-derived inactivated subunit, cell-derived inactivated subunit, egg-derived inactivated split virion). We excluded

Figure 4

Phylogenetic tree I-MOVE multicentre case–control study, Europe, influenza season 2014/15 (week 41/2014-week 19/2015)

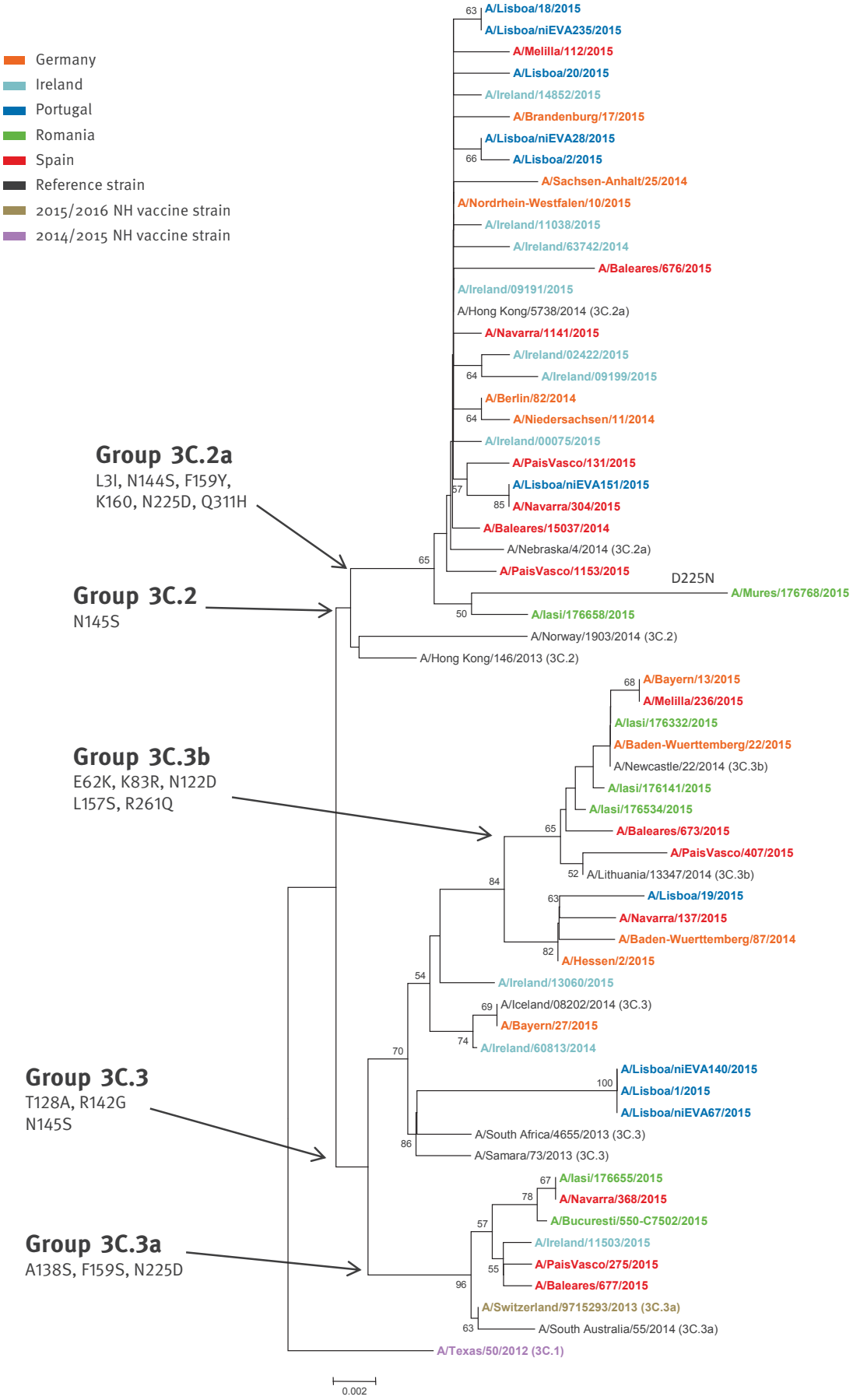


Table 1 a

Details of influenza haemagglutinin sequences obtained from GISAID used in the phylogenetic analysis, I-MOVE multicentre case–control study, Europe, influenza season 2014/15 (week 41/2014-week 19/2015)

Segment ID	Segment	Country	Collection date	Isolate name	Originating Laboratory	Submitting Laboratory	Authors
I-MOVE sequences							
EPI568197	HA	Germany	2 Feb 2015	A/Bayern/27/2015	NA	Robert Koch Institute	Wedde, M; Schweiger, S
EPI568195	HA		28 Jan 2015	A/Brandenburg/17/2015			
EPI566844	HA		19 Jan 2015	A/Bayern/13/2015			
EPI566843	HA		26 Jan 2015	A/Baden-Wuerttemberg/22/2015			
EPI566664	HA		9 Jan 2015	A/Nordrhein-Westfalen/10/2015			
EPI566662	HA		20 Jan 2015	A/Hessen/2/2015			
EPI566657	HA		22 Dec 2014	A/Sachsen-Anhalt/25/2014			
EPI562792	HA		22 Dec 2014	A/Baden-Wuerttemberg/87/2014			
EPI562791	HA		18 Dec 2014	A/Berlin/82/2014			
EPI562793	HA		24 Dec 2014	A/Niedersachsen/11/2014			
EPI599601	HA	Ireland	2 Mar 2015	A/Ireland/14852/2015	National Virus Reference Laboratory	National Virus Reference Laboratory	Dunford, L
EPI599599	HA		17 Feb 2015	A/Ireland/13060/2015			
EPI599597	HA		13 Feb 2015	A/Ireland/11503/2015			
EPI599594	HA		9 Feb 2015	A/Ireland/09191/2015			
EPI599593	HA		13 Jan 2015	A/Ireland/02422/2015			
EPI582398	HA		13 Feb 2015	A/Ireland/11038/2015			
EPI582390	HA		9 Feb 2015	A/Ireland/09199/2015			
EPI582379	HA		25 Nov 2014	A/Ireland/60813/2014			
EPI555113	HA		12 Dec 2014	A/Ireland/63742/2014			
EPI582380	HA		22 Dec 2014	A/Ireland/00075/2015			
EPI583766	HA	Portugal	3 Mar 2015	A/Lisboa/20/2015	Instituto Nacional de Saude	INSA National Institute of Health Portugal	Guiomar, R;Pechirra, P; Cristóvão, P; Costa, I
EPI583765	HA		20 Feb 2015	A/Lisboa/19/2015			
EPI583762	HA		16 Feb 2015	A/Lisboa/niEVA235/2015			
EPI583761	HA		6 Feb 20150	A/Lisboa/18/2015			
EPI583759	HA		22 Jan 2015	A/Lisboa/niEVA151/2015			
EPI583741	HA		29 Jan 2015	A/Lisboa/2/2015			
EPI583740	HA		27 Jan 2015	A/Lisboa/1/2015			
EPI565347	HA		16 Jan 2015	A/Lisboa/niEVA140/2015			
EPI558632	HA		2 Jan 2015	A/Lisboa/niEVA67/2015			
EPI558621	HA		30 Dec 2014	A/Lisboa/niEVA28/2015			
EPI599624	HA	Romania	11 Feb 2015	A/Bucuresti/550-C7502/2015	Cantacuzino Institute	Cantacuzino Institute	NA
EPI599678	HA		19 Jan 2015	A/Iasi/176332/2015			
EPI599698	HA		22 Jan 2015	A/Iasi/176534/2015			
EPI600298	HA		23 Jan 2015	A/Iasi/176655/2015			
EPI599769	HA		26 Jan 2015	A/Iasi/176658/2015			
EPI599770	HA		26 Jan 2015	A/Mures/176768/2015			
EPI599771	HA		13 Jan 2015	A/Iasi/176141/2015			
EPI566948	HA	Spain	3 Feb 2015	A/Baleares/676/2015	Servicio de Microbiología Hospital Universitario Son Espases	Instituto de Salud Carlos III	Pozo,F Calderon,A; Gonzalez -Esguevillas,M; Molinero,M; Casas,I
EPI616537	HA		10 Mar 2015	A/Navarra/1141/2015			
EPI616553	HA		10 Mar 2015	A/PaisVasco/1153/2015			
EPI559629	HA		17 Jan 2015	A/Melilla/236/2015			
EPI557585	HA		12 Jan 2015	A/Melilla/112/2015			
EPI616494	HA		3 Feb 2015	A/Baleares/677/2015			
EPI616493	HA		3 Feb 2015	A/Baleares/673/2015			
EPI557566	HA		13 Dec 2014	A/Baleares/15037/2014			
EPI566285	HA		21 Jan 2015	A/Navarra/368/2015	Servicio de Microbiología Complejo Hospitalario de Navarra		
EPI559633	HA		12 Jan 2015	A/Navarra/137/2015			
EPI567981	HA		23 Jan 2015	A/PaisVasco/407/2015			
EPI566296	HA		15 Jan 2015	A/PaisVasco/275/2015			
EPI566975	HA		12 Jan 2015	A/PaisVasco/131/2015			
EPI566282	HA		19 Jan 2015	A/Navarra/304/2015			

GISAID: Global Initiative on Sharing Avian Influenza Data.

Table 1 b

Details of influenza haemagglutinin sequences obtained from GISAID used in the phylogenetic analysis, I-MOVE multicentre case–control study, Europe, influenza season 2014/15 (week 41/2014-week 19/2015)

Segment ID	Segment	Country	Collection date	Isolate name	Originating Laboratory	Submitting Laboratory	Authors
I-MOVE sequences							
Reference sequences							
EPI398417	HA	United States	15 Apr 2012	A/Texas/50/2012	Texas Department of State Health Services-Laboratory Services	Centers for Disease Control and Prevention	NA
EPI460558	HA	Russian Federation	12 Mar 2013	A/Samara/73/2013	WHO National Influenza Centre Russian Federation	National Institute for Medical Research	
EPI696965	HA		29 Jan 2015	A/South Australia/55/2014 (14/226)	NA	National Institute for Biological Standards and Control (NIBSC)	Nicolson, C
EPI466802	HA	South Africa	25 Jun 2013	A/South Africa/4655/2013	Sandringham, National Institute for Communicable D	National Institute for Medical Research	NA
EPI536340	HA	Iceland	10 Jun 2014	A/Iceland/08202/2014	Landspitali - University Hospital		
EPI539598	HA	Lithuania	8 May 2014	A/Lithuania/13347/2014	Lithuanian AIDS Center Laboratory		
EPI541459	HA	Australia	16 Jun 2014	A/Newcastle/22/2014	WHO Collaborating Centre for Reference and Research on Influenza		
EPI426061	HA	Hong Kong (SAR)	11 Jan 2013	A/Hong Kong/146/2013	Government Virus Unit		
EPI539806	HA	Hong Kong (SAR)	30 Apr 2014	A/Hong Kong/5738/2014			
EPI539619	HA	United States	11 Mar 2014	A/Nebraska/4/2014	Centers for Disease Control and Prevention		
EPI530687	HA	Switzerland	6 Dec 20130	A/Switzerland/9715293/2013	Hopital Cantonal Universitaire de Geneve		

GISAID: Global Initiative on Sharing Avian Influenza Data.

study sites from the vaccine type analysis, where the given type of vaccine was not available.

We conducted four sensitivity analyses (i) restricting the study to patients swabbed less than 4 days after symptom onset, (ii) restricting to the population targeted for vaccination as defined in each country [23] (iii) excluding patients vaccinated < 15 days after symptom onset, (iv) calculating adjusted VE using a two-stage model using random effects.

The respective country's National Influenza Reference Laboratories tested swab specimens for influenza by real-time RT-PCR assays. In Spain, other laboratories participating in the National Influenza Sentinel Surveillance System tested specimens. In each study site, a non-random selection of positive specimens or isolated viruses from positive specimens were subsequently sent to the corresponding National Influenza Centre, where influenza diagnosis was confirmed and viruses characterised either by sequencing the HA1 coding portion of the haemagglutinin gene (genetic characterisation) or by haemagglutination inhibition (antigenic characterisation). The criteria to select the specimens for genetic and antigenic characterisation varied by study site.

For the I-MOVE pooled analysis, the Spanish and Portuguese National Influenza Centres analysed the nt and amino acid sequences of the HA1 coding portion of the haemagglutinin gene and used the neighbour-joining method and the Kimura 2-parameter nt substitution model for phylogenetic analysis. A phylogenetic tree was constructed with a bootstrap analysis of 500 replicates (values above 50 are shown) using MEGA software version 6 (Tamura, Stecher, Peterson, Filipski,

and Kumar 2013). HA sequences from reference strains used in the phylogenetic analysis were obtained from the EpiFlu database of the Global Initiative on Sharing Avian Influenza Data (GISAID) (Table 1).

Results

Within the I-MOVE multicentre case–control study, the start of country-specific study periods ranged from week 41, 2014 (Germany) to week 3, 2015 (Poland), and the end from week 13, 2015 (Portugal) to week 19, 2015 (Germany). Study period duration ranged from 14 (Poland) to 31 (Germany) weeks.

Among the 7,992 ILI patients recruited, 6,579 ILI patients met the eligibility criteria including 3,142 testing negative for all influenza viruses. For the influenza type/subtype-specific analysis datasets, we included 1,828 influenza A(H3N2), 1,038 influenza B, 539 influenza A(H1N1)pdm09 (Figure 1).

The median onset date was 1 February for A(H1N1) pdm09, 1 February for A(H3N2), and 20 February for B cases (Figure 2). Forty-one percent of A(H3N2) cases were recruited in Germany, 44% of A(H1N1)pdm09 in Italy and 30% of B cases in Spain.

The median age was higher in influenza B cases (39 years) compared with influenza A(H3N2) and A(H1N1) cases (28 and 30 years respectively) and controls (31 years).

The proportion of patients swabbed more than three days after ILI onset was 15.9% among controls, and 10.3%, 13.5% and 15.9% among A(H3N2), A(H1N1) pdm09 and B cases respectively.

The proportion of patients belonging to the target group for vaccination, or with at least one chronic condition or with at least one hospitalisation in the previous 12 months was similar between influenza A(H3N2), A(H1N1)pdm09, B cases and controls.

Nine percent of controls, and 11%, 5% and 6% of A(H3N2), A(H1N1)pdm09 and B cases had received both the 2013/14 and the 2014/15 vaccines.

Of the 735 vaccinated individuals, 620 (84%) had information on the vaccine type received; they were vaccinated with ten different brands. By vaccine type, 40% had received egg-derived inactivated subunit (used in all sites except in Hungary and Italy), 33% egg-derived inactivated split virion (used in all sites except in Ireland and Romania), 21% adjuvanted (used in Germany, Hungary, Italy and Spain) and 5% cell-derived inactivated subunit vaccines (used in Germany and Spain).

After excluding patients with missing information (n = 833; 7%), we included 4,491, 2,920 and 3,730 patients in the complete case analysis of VE against influenza A(H3N2), A(H1N1)pdm09 and B respectively (Figure 1).

The I^2 was < 50% ($p > 0.05$) when assessing crude type/subtype specific VE by study site and age group. Sample size among the 0–14 year-olds for the A(H1N1) pdm09 analysis was too small to carry out tests for heterogeneity. When assessing crude VE against A(H3N2) by study site among the target group for vaccination, the I^2 was 61.5% ($p = 0.016$).

Influenza A(H3N2)

The overall adjusted VE against influenza A(H3N2) was 14.4% (95% CI: -6.3 to 31.0) (Table 3).

Adjusted VE was 20.7% (95% CI: -22.3 to 48.5) among the 0–14 year olds, 10.9% (95% CI: -30.8 to 39.3) among the 15–59 year olds and 15.8% (95% CI: -20.2 to 41.0) among those ≥60 years. By vaccine type, the adjusted VE point estimates were lower for cell-derived inactivated subunit vaccines (-9.3%) compared with egg-derived inactivated subunit, egg-derived inactivated split virion, and adjuvanted vaccines (10.9%, 18.6% and 14.0% respectively) (Table 4).

The adjusted VE was 43.7% (95% CI: 15.3 to 62.5) among those vaccinated in 2014/15 only, 0.0% (95%CI: -50.7 to 33.7) among those vaccinated in 2013/14 only, and -5.2% (95%CI: -34.3 to 17.6) among those vaccinated in both seasons (Table 4, Figure 3).

The overall adjusted VE point estimate was similar to the adjusted VE among those swabbed less than 4 days of symptom onset (17.4%) and to the adjusted VE excluding individuals vaccinated less than 15 days after symptom onset (13.7%). The adjusted VE point estimate was higher when restricting the analysis to the target population (26.2%) (Table 2). The adjusted VE estimates using a two-stage random effects model were similar (within 6 % points) to the one-stage pooled analysis VE for all population and restricted to the target group for vaccination (Table 2). The two-stage VE point estimate in the ≥60 year- olds was 10% higher than the one-stage VE but three study sites were excluded from the two-stage analysis due to their limited sample size.

One hundred and fourteen (6%) of the 1,828 A(H3N2) viruses included in the analysis were genetically or antigenically characterised. Seventy-five viruses of the 114 (66%) were antigenically distinct from the vaccine virus A/Texas/50/2012: 58 belonged to clade 3C.2a, represented by A/HongKong/5738/2014, and 17 belonged to clade 3C.3a represented by A/Switzerland/9715293/2013 (Table 5).

Of the 114 characterised A(H3N2) viruses, 107 (94%) were sequenced. Compared with A/Texas/50/2012, 17 viruses had the T128A, R142G and N145S mutations that define the group 3.C represented by A/Samara/73/2013. Eight viruses had in addition the

Table 2

Details for influenza, A(H3N2), A(H1N1)pdm09 and influenza B cases and controls, I-MOVE multicentre case–control study, Europe, influenza season 2014/15 (week 41/2014-week 19/2015) (n=6,524[a])

Variables	Number of test-negative controls /total n(%) (n=3,142) [b]	Number of influenza A(H3N2) cases /total n(%) (n=1,828)[c]	Number of influenza A(H1N1) pdm09 /total n(%) (n=1,038)[d]	Number of influenza B cases /total n(%) (n=539) [c,d]
Median age (years)	31.0	28.0	30.0	39.0
Missing	5	1	1	0
Age groups				
0–4 years	620/3,137 (19.8)	212/1,827 (11.6)	136/538 (25.3)	62/1,038 (6)
5–14 years	459/3,137 (14.6)	451/1,827 (24.7)	85/538 (15.8)	219/1,038 (21.1)
15–59 years	1,539/3,137 (49.1)	885/1,827 (48.4)	256/538 (47.6)	619/1,038 (59.6)
≥60 years	519/3,137 (16.5)	279/1,827 (15.3)	61/538 (11.3)	138/1,038 (13.3)
Missing	5	1	1	0
Sex				
Female	1,610/3,132 (51.4)	945/1,825 (51.8)	283/539 (52.5)	556/1,037 (53.6)
Missing	10	3	0	1
Days between onset of symptoms and swabbing				
0	254/3,142 (8.1)	128/1,828 (7)	55/539 (10.2)	32/1,038 (3.1)
1	1,076/3,142 (34.2)	662/1,828 (36.2)	206/539 (38.2)	286/1,038 (27.6)
2	816/3,142 (26)	574/1,828 (31.4)	128/539 (23.7)	317/1,038 (30.5)
3	497/3,142 (15.8)	275/1,828 (15)	77/539 (14.3)	238/1,038 (22.9)
4–7	499/3,142 (15.9)	189/1,828 (10.3)	73/539 (13.5)	165/1,038 (15.9)
Seasonal vaccination, 2014/15e	392/2,978 (13.2)	228/1,759 (13.0)	36/522 (6.9)	75/1,010 (7.4)
Missing	164	69	17	28
Previous season influenza vaccination				
Not vaccinated or vaccinated<15 days before onset	2,432/2,918 (83.3)	1,461/1,733 (84.3)	464/515 (90.1)	901/1,001 (90)
Current season vaccination only	98/2,918 (3.4)	41/1,733 (2.4)	10/515 (1.9)	14/1,001 (1.4)
Previous season vaccination only	113/2,918 (3.9)	47/1,733 (2.7)	15/515 (2.9)	27/1,001 (2.7)
Current and previous season vaccination	275/2,918 (9.4)	1,84/1,733 (10.6)	26/515 (5.0)	59/1,001 (5.9)
Missing	224	95	24	37
2014/15 vaccine type				
Not vaccinated or vaccinated<15 days before onset	2,586/2,978 (82.3)	1,531/1,759 (83.8)	486/522 (90.2)	935/1,010 (90.1)
Egg-derived inactivated subunit	124/2,978 (3.9)	89/1,759 (4.9)	10/522 (1.9)	27/1,010 (2.6)
Egg-derived inactivated split virion	115/2,978 (3.7)	56/1,759 (3.1)	16/522 (3)	19/1,010 (1.8)
Adjuvanted	81/2,978 (2.6)	38/1,759 (2.1)	3/522 (0.6)	8/1,010 (0.8)
Cell- derived inactivated subunit	10/2,978 (0.3)	13/1,759 (0.7)	0/522 (0)	7/1,010 (0.7)
Unknown vaccine type	62/2,978 (2)	32/1,759 (1.8)	7/522 (1.3)	14/1,010 (1.3)
Missing vaccination status or date	164	69	17	28
At least one chronic condition	661/3,024 (21.9)	384/1,776 (21.6)	110/525 (21.0)	216/1,023 (21.1)
Missing	118	52	14	15
At least one hospitalisation in the previous 12 months for chronic conditions	56/3,100 (1.8)	25/1,806 (1.4)	7/534 (1.3)	23/1,033 (2.2)
Missing	42	22	5	5
Belongs to target group for vaccination	902/3,069 (29.4)	511/1,801 (28.4)	141/530 (26.6)	301/1,029 (29.3)
Missing	73	27	9	9
Study sites				
Germany	1,472/3,142 (46.8)	741/1,828 (40.5)	185/539 (34.3)	268/1,038 (25.8)
Ireland	109/3,142 (3.5)	102/1,828 (5.6)	11/539 (2)	57/1,038 (5.5)
Hungary	379/3,142 (12.1)	232/1,828 (12.7)	32/539 (5.9)	42/1,038 (4)
Portugal	102/3,142 (3.2)	45/1,828 (2.5)	0/539 (0)	98/1,038 (9.4)
Italy	594/3,142 (18.9)	229/1,828 (12.5)	237/539 (44)	123/1,038 (11.8)
Poland	77/3,142 (2.5)	18/1,828 (1)	21/539 (3.9)	70/1,038 (6.7)
Romania	76/3,142 (2.4)	80/1,828 (4.4)	43/539 (8)	73/1,038 (7)
Spain	333/3,142 (10.6)	381/1,828 (20.8)	10/539 (1.9)	307/1,038 (29.6)

I-MOVE: Influenza Monitoring Vaccine Effectiveness in Europe.

[a] This includes 15 influenza B+A(H3N2) co-infections and 8 influenza B+A(H1N1)pdm09 co-infections. Note that numbers of cases come from influenza type/subtype specific databases. Some cases are excluded due to their restriction criteria. Any influenza A non-typed cases are dropped from analysis.

[b] Controls from 'any influenza' analysis used.

[c] Includes 15 influenza B+A(H3N2) co-infections.

[d] Includes 8 influenza B+A(H1N1)pdm09 co-infections.

[e] Vaccination more than 14 days before onset of influenza like illness symptoms.

Table 3

Pooled crude and adjusted seasonal vaccine effectiveness against laboratory-confirmed influenza by influenza type/subtype, overall and by age groups, I-MOVE multicentre case–control study, Europe, influenza season 2014/15 (week 41/2014-week 19/2015)

Type/subtype	Analysis scenario		N[a,b]	Cases;vaccinated/Controls; vaccinated[a,b]	Crude VE[a,c]	95% CI	Adjusted VE	95% CI
A(H3N2)	1-stage pooled analysis[d]	All ages	4,491	1,723;225/2,768;365	-1.9	-22.2 to 15.1	14.4	-6.3 to 31.0
		0–14 years	1,505	607;54/898;64	-38.4	-103.5 to 5.9	20.7	-22.3 to 48.5
		15–59 years	2,245	846;57/1,399;91	-2.2	-45.3 to 28.1	10.9	-30.8 to 39.3
		≥60 years	741	270;114/471;210	7.3	-26.9 to 32.2	15.8	-20.2 to 41.0
		Target group for vaccination	1,287	483;155 / 804;276	10.9	-14.5 to 30.6	26.2	1.6 to 44.7
		Vaccinated <15 days excluded	4,475	1,718;225/2,757;365	-1.8	-22.2 to 15.1	13.7	-7.2 to 30.5
		Restricted delay onset and swabbing<4 days	3,869	1,543;196/2,326;280	-10.1	-34.4 to 9.8	17.4	-4.6 to 34.8
	2-stage pooled analysis	All ages	4,503	1,724;225/2,779;366	-0.6	-31.2 to 22.8	9.0	-28.2 to 35.4
		0–14[e] years	1,418	564;54/853;63	-42.2	-109.2 to 3.3	22.9	-20.7 to 50.8
		15–59[f] years	2,192	853;57/1,357;88	-6.6	-53.2 to 25.8	12.3	-31.6 to 41.5
		≥60[g] years	678	254;108/424;187	11.3	-24.9 to 37.1	25.5	-24.5 to 55.4
		Target group for vaccination[h]	1,240	473;153/767;274	6.4	-43.2 to 38.9	20.7	-32.5 to 52.5
A(H1N1)pdm09	1-stage pooled analysis[i]	All ages	2,920	515;36/2,405;314	53.7	33.1 to 68.0	54.2	31.2 to 69.6
		0–14 years	1,023	211;8/812;63	59.9	13.4 to 81.5	73.1	39.6 to 88.1
		15–59 years	1,436	245;8/1191;75	47.5	-13.1 to 75.6	59.7	10.9 to 81.8
		≥60 years	451	59;20/392;171	22.4	-44.4 to 58.4	22.4	-44.4 to 58.4
		Target group for vaccination	832	138;26/694;232	53.8	26.0 to 71.2	53.6	22.1 to 72.3
		Vaccinated<15 days excluded	2,914	515;36/2,399;314	53.9	33.3 to 68.1	54.5	31.6 to 69.7
		Restricted delay onset and swabbing<4 days	2,471	443;26/2,028;242	57.8	35.3 to 72.5	61.0	37.7 to 75.6
	2-stage pooled analysis	All ages[j]	2,650	494;34/2,156;285	53.6	20.6 to 72.9	53.5	27.8 to 70.1
		0–14[k] years	916	196;7/720;59	59.5	-79.6 to 90.9	71.6	20.5 to 89.9
		15–59[l] years	941	195;7/746;52	35.4	-51.3 to 72.4	51.8	-15.9 to 79.9
		≥60[m] years	290	41;18/249;120	15.8	-65.3 to 57.1	NA	NA
		Target group for vaccination[n]	536	105;22/431;160	53.8	22.3 to 72.5	58.4	10.7 to 80.6
Influenza B	1-stage pooled analysis	All ages	3,730	1,001;74 / 2,729;362	47.9	31.3 to 60.4	48.0	28.9 to 61.9
		0–14 years	1,143	269;11 / 874;62	37.8	-23.2 to 68.6	62.1	14.9 to 83.1
		15–59 years	1,986	602;29 / 1,384;94	29.6	-10.3 to 55.0	41.4	6.2 to 63.4
		≥60years	601	130;34 / 471;206	54.4	25.8 to 72.0	50.4	14.6 to 71.2
		Target group for vaccination	1,083	290;56 / 793;273	54.6	35.2 to 68.2	49.8	26.2 to 65.9
		Vaccinated <15 days excluded	3,719	998;74/2,721;362	47.8	31.3 to 60.4	47.8	28.6 to 61.8
		Restricted delay onset and swabbing <4 days	3,132	841;63/2,291;278	41.8	21.3 to 57.0	44.4	21.8 to 60.5
	2-stage pooled analysis	All ages	3,734	1,003;74/2,731;363	48.9	25.3 to 65.0	51.5	26.8 to 61.8
		0–14[p] years	1,057	230;12/827;61	29.5	-41.3 to 64.8	47.5	-15 to 76.0
		15–59 years	1,995	603;29/1,392;96	28.1	-17.1 to 55.9	43.2	5.2 to 66.0
		≥60[q] years	611	132;34/479;208	53.5	24.1 to 71.5	54.1	22.4 to 72.8
		Target group for vaccination[r]	1,057	293;56/764;266	54.9	27.2 to 72.0	56.0	26.2 to 73.8

CI: confidence interval; DE: Germany; ES: Spain; HU: Hungary; IE: Ireland; I-MOVE: Influenza Monitoring Vaccine Effectiveness in Europe; IT: Italy; PL: Poland; PT: Portugal; RO: Romania; VE: vaccine effectiveness.

[a] Based on the complete case analysis: records with missing age, sex, chronic condition, vaccination status are dropped.

[b] Totals may differ between one-stage and two-stage models, as adjustment at study site-level may vary to the one-stage pooled model adjustment, resulting in different missing data dropped depending on included covariates. In addition different numbers of study sites may be included in each analysis due to sample size issues.

[c] Crude VE adjusted by study site.

[d] Data adjusted for age (restricted cubic spline), onset date (restricted cubic spline), sex, chronic condition and study site. Exceptions are A(H3N2) all ages, where age groups (0–4, 5–14, 15–59 and ≥60 years) are used instead of restricted cubic splines.

[e] Study sites include DE, ES, IT. HU not included in the 0–14 year old analysis, as no patients included aged <18 years. Sample size too low for IE, PT and RO.

[f] Study sites include DE, ES, HU, IE, IT, PT, RO. Sample size too low for PL. Crude VE for RO used in adjusted estimate, due to low sample size.

[g] Study sites include DE, ES, HU, IT, RO. IE, PL and PT not included due to low sample size. Crude VE for RO used in adjusted estimate, due to low sample size.

[h] Study sites include DE, ES, IE, IT, PL, PT, RO. HU not included in the 0–14 year old analysis, as no patients included aged <18 years.

[i] Data adjusted for age (restricted cubic spline), onset date (restricted cubic spline), sex, chronic condition and study site. Exceptions the A(H1N1)pdm09 analysis among the elderly, where data are adjusted for age (restricted cubic spline), onset date (restricted cubic spline), and study site only.

[j] Study sites include DE, HU, IE, IT, RO, PL. ES and IE dropped from analysis due to small sample size.

[k] Study sites include DE, IT. ES, IE, PL, RO not included as sample size too low. HU not included in the 0–14 year old analysis, as no patients included aged<18 years.

[l] Study sites include DE, IT, RO. ES, HU, IE and PL not included as sample size too small. Crude VE for RO used in adjusted estimate, due to low sample size.

[m] Study sites include DE, IT. ES, HU, IE, PL and RO not included as sample size too small. Only crude VE available, due to low sample size.

[n] Study sites include DE, IT, RO. ES, HU, IE and PL not included as sample size too small. Crude VE for RO used in adjusted estimate, due to low sample size.

[o] Data adjusted for age (restricted cubic spline), onset date (restricted cubic spline), sex, chronic condition and study site. Exceptions the B analysis among the elderly, where data are adjusted for age (restricted cubic spline), onset date (restricted cubic spline), and study site only.

[p] Study sites include DE, ES, IT. IE, PL, PT and RO not included as sample size too low. HU not included in the 0–14 year old analysis, as no patients included aged<18 years.

[q] Study sites include DE, ES, HU, IE, IT, PL, PT, RO. Crude VE for DE, HU, IE, PL and RO due to low sample size.

[r] Study sites include DE, ES, HU, IE, IT, PL, PT, RO. Crude VE for HU, IE and RO due to low sample size.

mutations G5E and N31S. Twenty viruses belonged to the group 3C.3b represented by A/Newcastle/22/2014 and characterised by T128A, R142G, N145S, E62K, K83R, N122D, L157S and R261Q mutations. Seven of these presented an additional amino acid change Q197H at the antigenic site B (Figure 4).

Twelve viruses belonged to the group 3C.3a that harbours the T128A, R142G, A138S, N145S, F159S and N225D mutations. Nine of them had an extra mutation K276N at the antigenic site C. Fifty-eight viruses belonged to group 3C.2a and the only mutations identified were L3I, N144S, N145S, F159Y, K160T, N225D and Q311H - amino acid mutations that define the group.

Influenza A(H1N1)pdm09

The overall adjusted VE against influenza A(H1N1) pdm09 was 54.2% (95% CI: 31.2 to 69.6) (Table 3).The adjusted VE was 73.1% (95% CI: 39.6 to 88.1) among the 0–14 year olds, 59.7% (95% CI: 10.9 to 81.8) among the 15–59 year olds and 22.4% (95% CI: -44.4 to 58.4) among those ≥60 years of age.

By vaccine type, the adjusted VE point estimate was higher for the adjuvanted vaccine (79.8%) than for the egg-derived inactivated subunit and the inactivated split virion vaccines (53.0% and 51.5% respectively). We could not compute the VE for the cell-derived inactivated subunit due to small numbers (7 controls vaccinated and no cases vaccinated) (Table 4).

The adjusted VE point estimate was lower (-1.9%) among those vaccinated in 2013/14 only compared with those vaccinated in 2014/15 only (47.2%) and to those vaccinated in both seasons (52.7%) (Table 4).

The overall adjusted VE point estimate did not vary when restricting the analysis to the target group for vaccination (53.6%), when excluding those vaccinated<15 days (54.5%) before symptom onset and when using a two-stage pooled model (53.5%). It was 61.0% when restricted to those swabbed less than 4 days of symptom onset (Table 3).

Of the 539 A(H1N1)pdm09 viruses, 24 (4%) were genetically characterised and all belonged to the group 6B defined by the amino acid substitutions D97N, K163Q, S185T, S203T, A256T and K283E compared with A/California/07/2009.

Influenza B

The overall adjusted VE against influenza B was 48.0% (95% CI: 28.9 to 61.9). The adjusted VE was 62.1% (95% CI: 14.9 to 83.1) among the 0–14 year olds, 41.4% (95% CI: 6.2 to 63.4) among the 15–59 year olds and 50.4% (95% CI: 14.6 to 71.2) among those ≥60 years old (Table 3).

By vaccine type, the adjusted VE point estimates were lower for cell-derived inactivated subunit vaccines (16.0%) than for egg-derived subunit, split virion and adjuvanted vaccines (52.4%, 60.1%, 51.9% respectively) (Table 4).

The adjusted VE point estimate was lower among those vaccinated only in 2013/14 (1.7%) than among those vaccinated only in 2014/15 (59.4%) or among those vaccinated in both seasons (43.8%) (Table 4).

There was less than 9% absolute difference between the overall adjusted VE point estimates and the VE in all sensitivity analyses (Table 3). The two-stage VE point estimate in the 0–14 years old was 15% lower than the one-stage VE point estimate but five study sites were excluded from the two-stage analysis due to their limited sample size.

Among 746 cases for which the lineage was available, 740 (99.2%) were Yamagata and six Victoria.

One hundred and fifty-three (15%) of the 1,038 B viruses were characterised: 151 B Yamagata and two B Victoria viruses. Of the 151 B Yamagata lineage viruses genetically characterised, 148 (98%) belonged to B/Phuket/3073/2013, clade 3 and three to B/Massachusetts/02/2012. The two B Victoria viruses genetically characterised belonged to B/Brisbane/60/2008 (1A).

Discussion

The results of the I-MOVE multicentre case–control study suggest a low 2014/15 influenza VE against medically attended ILI due to A(H3N2) and a moderate VE against medically attended ILI due to A(H1N1)pdm09 or B.

The sample size of the I-MOVE multicentre case–control study for the 2014/15 season was one of the largest since 2008/09. We could estimate VE against the three circulating viruses. However, with the low influenza vaccination coverage in the participating sites, we still have limited statistical power for some subgroup analyses that provide important information for public health action like VE by previous vaccination or VE by type of vaccine. The current sample size is still too small to measure VE by vaccine product.

Measuring VE by study sites was not among the objectives of our multicentre study. In addition, as in previous seasons, study sites, sample size pending, are publishing their own results. However, even if not statistically significant, VE may differ between study sites. Differences in site-specific adjusted VE may be explained, among other factors, by variability due to the limited number of samples, unknown residual confounding, or different vaccines used. In future seasons we are confident that, with more resources, sample sizes should increase allowing for better adjustment and stratification including by vaccine brand.

Integrating virological and epidemiological information is essential to interpret VE estimates [5]. For the

Table 4

Pooled crude and adjusted seasonal vaccine effectiveness against laboratory- confirmed influenza by influenza type/subtype, by vaccine type and by influenza vaccination status in 2013/14, I-MOVE multicentre case–control study, Europe, influenza season 2014/15 (week 41/2014-week 19/2015)

Influenza type/ subtype		Vaccine type	N	Cases/controls	Crude VE[a,b]	95% CI	Adjusted VE[c]	95% CI
A(H3N2)	By vaccine type	Unvaccinated	3,901	1,498/2,403	Ref	NA	Ref	NA
		Egg-derived inactivated subunit	205	88/117	-5.7	-41.7 to 21.2	10.9	-24.3 to –36.1
		Egg-derived inactivated split virion	164	56/108	-0.4	-41.2 to 28.6	18.6	-17.4 to 43.5
		Adjuvanted	116	38/78	11.8	-32.7 to 41.4	14.0	-34.1 to 44.9
		Cell-Derived inactivated subunit	23	13/10	-15.3	-167.0 to 50.2	-9.3	-159.1 to 53.9
		Unknown	82	30/52	-12.0	-77.1 to 29.2	21.3	-29.7 to 52.3
	By previous vaccination	Unvaccinated in both seasons	3,697	1,434/2,263	Ref	NA	Ref	NA
		Vaccinated in 2014/15 only	133	41/92	29.8	-2.7 to 52.0	43.7	15.3 to 62.5
		Vaccinated in 2013/14 only	147	43/104	28.2	-3.4 to 50.2	0.0	-50.7 to 33.7
		Vaccinated in both seasons	436	181/255	-16.4	-43.1 to 5.3	-5.2	-34.3 to 17.6
A(H1N1)pdm09	By vaccine type	Unvaccinated	2,570	479/2,091	Ref	NA	Ref	NA
		Egg-derived inactivated subunit	113	10/103	47.1	-4.5 to 73.2	53.0	4.1 to 76.9
		Egg-derived inactivated split virion	104	16/88	47.5	8.1 to 70.0	51.5	13.4 to 72.8
		Adjuvanted	73	3/70	84.4	49.3.to.95.2	79.8	31.0.to.94.1
		Cell-derived inactivated subunit	7	0/7	NA	NA	NA	NA
		Unknown	53	7/46	24.8	-70.7 to 66.8	35.3	-48.5 to 71.8
	By previous vaccination	Unvaccinated in both seasons	2,438	459/1,979	Ref	NA	Ref	NA
		Vaccinated in 2014/15 only	90	10/80	46.6	-5.8 to 73.0	47.2	-7.1 to 74.0
		Vaccinated in 2013/14 only	99	15/84	11.8	-56.8 to 50.4	-1.9	-86.2 to 44.2
		Vaccinated in both seasons	242	26/216	53.8	28.9 to 69.9	52.7	24.2 to 70.5
B	By vaccine type	Unvaccinated	3,294	927/2,367	Ref	NA	Ref	NA
		Egg-derived inactivated subunit	146	27/119	49.3	20.7 to 67.6	52.4	22.9 to 70.6
		Egg-derived Inactivated split virion	119	18/101	59.5	30.8 to 76.3	60.1	30.1 to 77.3
		Adjuvanted	86	8/78	51.3	-4.1 to 77.2	51.9	-6.2 to 78.2
		Cell-derived Inactivated subunit	17	7/10	22.5	-108.0 to 71.1	16.0	-129.9 to 69.3
		Unknown	68	14/54	25.0	-40.7 to 60.0	27.3	-40.2 to 62.3
	By previous vaccination	Unvaccinated in both seasons	3,127	894/2,233	Ref	NA	Ref	NA
		Vaccinated in 2014/15 only	107	14/93	61.1	29.8 to 78.4	59.4	25.1 to –78.0
		Vaccinated in 2013/14 only	128	26/102	20.3	-26.6 to 49.8	1.7	-61.8 to 40.3
		Vaccinated in both seasons	309	58/251	43.3	22.5 to 58.6	43.8	20.0 to 60.5

CI: confidence interval; Ref: reference; I-MOVE: Influenza Monitoring Vaccine Effectiveness in Europe; NA: not applicable; VE: vaccine effectiveness.

[a] Based on the complete case analysis: records with missing age, sex, chronic condition, vaccination status are dropped).

[b] Crude VE adjusted by study site.

[c] Data adjusted for age (restricted cubic spline or age group), onset date (restricted cubic spline), sex, chronic condition and study site.

Note: Egg-derived inactivated subunit vaccines used in DE, IE, PO, PT, RO, ES.

Egg-derived inactivated Split virion vaccines used in DE, HU, IT, PO, PT, ES.

Adjuvanted vaccines used in DE, HU, IT, ES.

Cell-derived inactivated subunit vaccines used in Germany, ES.

Table 5

Influenza A(H3N2), A(H1N1)pdm09, B Yamagata, B Victoria viruses characterised by clade and study site, I-MOVE multicentre case–control study, Europe, influenza season 2014/15 (week 41/2014-week 19/2015) (n=291)

Characterised viruses	Clade	Germany N	Hungary N	Ireland N	Portugal N	Romania N	Spain N	Total (%)
A(H3N2) (n=114)								
A/HongKong/5738/2014	3C.2a	12	NA	11	14	2	19	58 (51)
A/Switzerland/9715293/2013	3C.3a	NA	NA	1	NA	11	5	17 (15)
A/Samara/73/2013	3C.3	5	NA	3	4	3	4	19 (17)
A/Newcastle/22/2014	3C.3b	5	2	1	NA	3	9	20 (17)
Total A(H3N2)	NA	22	2	16	18	19	37	114 (100)
A(H1N1)pdm09 (n=24)								
A/SouthAfrica/3626/2013	6B	12	NA	5	2	5	NA	24 (100)
B Yamagata (n=151)								
B/Phuket/3073/2013	Clade 3	31	NA	5	56	28	28	148 (98)
B/Massachusetts/02/2012	Clade 2	NA	NA	NA	1	2	NA	3 (2)
Total B Yamagata	NA	31	NA	5	57	30	28	151 (100)
B Victoria (n=2)								
B/Brisbane/60/2008	NA	NA	NA	2	NA	NA	NA	2 (100)

NA: not applicable.

last two seasons, the I-MOVE multicentre case–control teams have made an effort to include genetic and antigenic results from a sample of the cases included in the study. However, the proportion of strains genetically and antigenically characterised (8.5%) is still low, and varied by site. Two study sites (Italy, Poland) could not provide results and some sites with a low number of cases characterised a higher proportion of viruses than sites with high number of cases. For instance, 11 of the 17 clade 3C.3a viruses characterised were from Romania, a site that contributed to only 4.4% of the A(H3N2) cases. In addition, the viruses characterised were selected according to virological surveillance objectives (e.g. selection of viruses from more severe cases, from vaccinated cases, etc.). Due to the non-random selection and the different proportion of viruses characterised we cannot exclude that the viruses characterised may not be representative of the viruses from cases included in the study. For the 2015/16 season, the I-MOVE multicentre case–control study will pilot a selection procedure aiming to provide a representative sample of viruses characterised. If resources are available, the number of viruses characterised should increase.

The VE against influenza A(H3N2) was low overall, by age group and among the target group for vaccination. Four different genetic clades of A(H3N2) viruses (3C.2a, 3C.3a, 3C.3 and 3C.3b) circulated in the eight countries participating in I-MOVE. The low VE are in concordance with the high proportion (66%) of 3C.2a and 3C.3a drifted viruses identified among those genetically characterised. Additional mutations were detected in the 3C.3 and 3C.3b influenza A(H3N2) viruses characterised but those are considered antigenically similar to the vaccine virus [13]. This season, estimates are similar to the VE against A(H3N2) we observed in 2011/12 and 2013/14 [8,9]. They are lower than the final 2014/15 VE against A(H3N2) reported in the UK even if the proportion of drifted virus among those genetically characterised are higher in UK than in our study [14]. VE against A(H3N2) was below 20% for all vaccine types with a lower point estimate for the cell-derived subunit vaccine. The effectiveness was lower in those vaccinated in both 2013/14 and 2014/15 than in those vaccinated only in the 2014/15 season. These observations are in line with the results of the 2014/15 early A(H3N2) VE estimates in Canada [5] and with those observed in previous studies [15-17]. They are congruent with the hypothesis that prior immunisation may decrease the effectiveness of the vaccine and that this negative interference is more important when the antigenic distance is small between successive vaccine components but large between vaccine and circulating strain [18]. These conditions were present in 2014/15 with an unchanged A(H3N2) vaccine component compared with the 2013/14 vaccine and with a mismatch between the vaccine and a high proportion of circulating strains. However, those results may be due to chance, or to bias. We need a much larger sample size to have higher precision in the estimates and to study the effect of prior vaccinations by age group. In our study, individuals vaccinated in both seasons are older than those vaccinated only in one season (median age 63 years and 50 years respectively). Unmeasured differences between individuals vaccinated in two consecutive seasons and those vaccinated only in one season may have affected the results. Previous vaccination was documented through GP records or patient self-reports and may be subject to error. Since neither the ILI patient nor the GPs knew if the patient was an influenza case we are confident that differential recall

did not bias the results. If the results were not due to bias or to chance, concurrent immunological studies will be essential to better understand the biological mechanism behind, and the role of natural vs vaccine-acquired immunity.

The VE estimates against influenza A(H1N1)pdm09 are similar to our results in previous seasons [7-9]. The laboratory results indicate that the strains isolated from study participants were similar to the A(H1N1) pdm09 component of the 2014/15 influenza vaccine. As in 2013/14, we observed a lower VE among the elderly and higher among those aged 0–14 years old, however sample sizes were small in the age group analyses. The VE point estimates of the adjuvanted vaccines were higher but the small sample size in the analysis does not allow a comparison of effectiveness between vaccine types.

The VE against influenza B ranged from 41% to 62% in the overall population and was 56% in the target group for vaccination. Our estimates are similar to those reported by the UK [14]. Nearly all viruses (99%) for which lineage was available were B/Yamagata and 98% of those characterised belonged to clade 3 that is antigenically similar to the vaccine virus. VE was similar by vaccine type with lower point estimate for cell-derived inactivated subunit vaccines but the sample size is too low to interpret this observed difference. The results suggested no effect of the 2013/14 vaccine and a slightly lower VE among those vaccinated in both seasons.

This is the third season we provide VE by vaccine type. A high proportion of vaccinated study participants (84%) had vaccine product documented. Even with one of the largest sample size since 2008/09, the numbers are still too low to measure adjusted VE by vaccine type and age group. The European Medicines Agency (EMA) requests that vaccine producers provide product-specific vaccine effectiveness [19]. Taking into account the high number of vaccine products and the low vaccination coverage in countries participating in the study [20] the sample size to measure VE by vaccine product with high precision has to be much larger and substantial additional resources are needed. In a survey among I-MOVE partners to assess the feasibility of conducting product-specific VE in Europe (data not shown) most experts considered that in terms of resources allocation, providing precise estimates early in the season, by age group, by previous vaccination were of higher priority than measuring VE by product.

In summary, the 2014/15 results suggest a moderate effectiveness against influenza A(H1N1)pdm09 and B. The low effectiveness of the influenza vaccines against A(H3N2) observed again this season underlines the need to improve the A(H3N2) component of the vaccine especially among the target group for vaccination. This would be even more important if the observed negative effect of previous vaccination was confirmed. Since A(H3N2) virus is generally associated with more severe disease in the elderly and high-risk groups [21,22] and the vaccine is less effective against this influenza subtype, in seasons of A(H3N2) circulation early antiviral treatment should be recommended in these groups [3,6].

The effect of previous vaccinations is one of the questions that I-MOVE and other influenza VE teams in the US, Canada and Australia started to raise some years ago [17,24-27]. This is an important issue that may impact vaccination policy in Europe. They need to be addressed through international collaboration, a multidisciplinary approach and with long-term scientific independent studies. The I-MOVE multicentre case–control study should continue to increase the sample size and to strengthen the virological component of the study to contribute to answer these questions.

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Conflict of interest

None declared.

Authors' contributions

All authors provided contribution to the research article and approved the final version.

Marta Valenciano, coordinated the I-MOVE multicentre case control study network, supervised the statistical analysis and interpretation of the results, led the writing of the research article.

Esther Kissling was responsible for the data management of the multicentre study, undertook the statistical analysis on which the research article is based, contributed to the writing of the research article

Marta Valenciano, Esther Kissling and Alain Moren were involved in the original methodological design

Annicka Reuss, Caterina Rizzo, Alin Gherasim, Judit Krisztina Horváth, Lisa Domegan, Daniela Pitigoi, Ausenda Machado, Iwona Anna Paradowska-Stankiewicz, Antonino Bella, Amparo Larrauri, Annamária Ferenczi, Joan O´Donell, Mihaela Lazar, Ausenda Machado, Monika Roberta Korczyńska, coordinated the corresponding national component of the I-MOVE study, contributed to the conception, design, acquisition and interpretation of the data.

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Letter to the editor: Is there a need for special treatment of refugees at hospital admission?

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To the editor: Two recent publications by Reinheimer et al. and Heudorf et al. in *Eurosurveillance*, provided data on multidrug-resistant bacteria obtained from screening of different refugee populations and concluded that additional screening or surveillance for refugees at hospital admission in Germany should be undertaken [1,2]. The high number of people currently migrating to Europe from disaster areas has sparked a debate, whether or not refugees should be screened at hospital admission for colonisation with multidrug-resistant bacteria to limit spread of antibiotic resistance within Europe. The possible negative consequences of screening and lacking data make this a more complex issue than it may seem at first.

The advantages of screening seem obvious. There is evidence that in some countries with a high emigration the prevalence of antimicrobial resistance in healthcare settings is high. In addition, international travel and migration are discussed as factors promoting the global spread of resistance [3]. One could also speculate that resistant pathogens may spread during the journey or within refugee camps, where hygiene is often poor and turnover of persons is high. However, evidence to base a decision for or against microbiological screening in refugees is largely lacking.

Little can be done in terms of decolonisation in case of multidrug-resistant Gram-negative bacteria (MDRGN), because they can colonise the human gut and become part of the intestinal flora. Therefore, good adherence to infection control precautions is essential to prevent the transmission of MDRGN in hospitals. In addition to standard precautions, isolation and barrier nursing are possible intervention strategies. However, these measures may be associated with poorer patient care [4] and higher cost. In the context of refugees it is also perceivable that targeted screening will result in stigmatisation.

Given the scarcity of available data in the scientific literature, the recently published articles by Reinheimer et al. and Heudorf et al. [1,2] are highly interesting. They show that in a sample of 143 adult refugees presenting at a German hospital as well as among 119 unaccompanied refugee minors, colonisation with extended spectrum beta-lactamase (ESBL)-producing bacteria is more frequent than in patients from general German population. Both articles suggest additional screening or surveillance for refugees at hospital admission in Germany. The Robert Koch Institute on the other hand does not recommend screening particularly for refugees in addition to what is recommended for everyone at hospital admission in Germany: if a patient had recent contact to the healthcare system of countries with high prevalence of antimicrobial resistance, screening for carbapenem-resistant bacteria and meticillin-resistant *Staphylococcus* aureus (MRSA) is recommended [5]. Reinheimer et al. calculated that according to current German guidelines [6], 32.9% of refugees in contrast to 10.9% of non-refugee patients would qualify for isolation in especially vulnerable settings, such as intensive care units (ICUs). This is misleading since not all of the patients in their sample had to be admitted to such settings.

While the high rate of colonisation with ESBL-producing bacteria among refugees is certainly worrisome and while current guidelines indeed recommend isolation in certain settings, such as in ICUs, screening for these pathogens is not recommended for any patient group in Germany [6]. In addition, colonisation with ESBL-producing bacteria may also be high in some German populations. For example a study among travellers returning to Germany showed colonisation with ESBL-producing *Escherichia coli* in 30% [3], which is similar to that seen among refugee minors [2]. Screening is, however, not recommended for travellers returning to Germany.

As described above screening for carbapenem-resistant bacteria is recommended for high risk groups [6]. Fortunately, carbapenem resistance was low in both studies, so that the need for targeted screening in this population under current German guidelines does not seem to be warranted. If, however, the proposal of an extension of current screening criteria was intended, Germans at high risk for colonisation with ESBL-producing bacteria needed to be included too. In the meantime strengthening standard hospital hygiene and providing translators for refugees to ensure good patient care and to identify those patients, who qualify for isolation and screening under current guidelines, may be more urgent.

Conflict of interest

None declared.

Authors' contributions

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Author's reply: Is there a need for special treatment of refugees at hospital admission?

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To the editor: We would like to thank Walter and colleagues for their comment [1] on our respective investigations published in Eurosurveillance [2,3]. While we agree with many of the statements made, we would nevertheless like to clarify the following points:

The Robert Koch Institute published recommendations for multidrug-resistant organisms (MDRO) screening of refugees on hospital admission already in October 2015 [4]. This document stated that according to the recommendations of the German Commission of Hospital Hygiene and Infection Prevention screening for multidrug-resistant organisms (MDRO) on hospital admission is necessary for patients coming from regions with high prevalence rates for MDRO, with previous contact to the health system in their country of origin or on route. Furthermore it points out that screening should encompass meticillin-resistant *Staphylococcus* aureus (MRSA) and carbapenem-resistant bacteria, only [5].

Infection control measures always represent a trade-off between patient safety and best medical treatment. We have shown that (i) the prevalence of MRSA is significantly higher in refugees (REF) (5.6%) than in a comparison group of resident population not admitted from a refugee accommodation (NREF) (1.2%) which itself justifies pre-emptive isolation, (ii) the prevalence of extended spectrum beta-lactamase (ESBL)-producing bacteria is significantly higher in REF which indeed has no infection control relevance, (iii) the prevalence of MDRGN with additional resistance to fluoroquinolones (so called 3MRGN in Germany) is significantly higher in REF which implies clearly consequences for hospital hygiene measures at least in special settings, e-g., intensive care units and (iv) the prevalence of carbapenem-resistant MDRGN strains (so called 4MRGN in Germany) in REF also, even if only slightly higher.

MDRO prevalence varies between distinct groups of patients and appropriate risk assessment has been established at Frankfurt University Hospital since five years e.g. for patients returning from high prevalence countries who have had contact with foreign health-care systems. Increased MRSA, and, possibly also increased 3MRGN rates justify screening procedures and isolation in certain risk groups. Identification of risk groups and introduction of adequate infection control measures are genuine duties of hospital infection control and are uncomfortable, may affect medical treatment and are certainly costly. However, we feel that our approach is necessary to ensure best medical practice and safety for all of our patients regardless of their country of origin and without stigmatisation.

Conflict of interest

None declared.

Authors' contributions

UH: literature work, writing. VK: literature work, writing.

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ECDC public consultation on the use of neuraminidase inhibitors

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On 17 February 2016, the European Centre for Disease Prevention and Control (ECDC), launched a public consultation on the use of oseltamivir and zanamivir.

Following a consultation with international public health experts, convened in Stockholm in February 2015 to review data presented in newly conducted systematic reviews and meta-analyses of clinical studies on influenza antivirals [1–3], an ECDC expert opinion was developed. This opinion has now been published on the ECDC website and is open for comments until 17 March 2016.

Oseltamivir and zanamivir, neuraminidase inhibitors, are currently authorised in the EU and the European Economic Area for treatment and prophylaxis of influenza disease. The drugs have been the subject of debate concerning their effectiveness and safety, and the appropriateness of stockpiling these drugs for use in future influenza pandemics has also been discussed.

Read more here.

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First European Union Laboratory Capability Monitoring System report published

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On 15 February, the European Centre for Disease Prevention and Control (ECDC) published the first European Union Laboratory Capability Monitoring System (EULabCap) report.

The EULabCap is a tool for assessing and monitoring the laboratory capacities and capabilities in the European Union/European Economic Area to underpin public health surveillance and assessment of risk posed by infectious disease. Furthermore, it gives an indication on the progression towards agreed upon practice standards and public health targets. This assessment aims at helping policymakers identify possible areas for action and evaluate the impact of capacity strengthening activities and health system reforms.

The tool combines 60 indicators (grouped in 12 targets each comprising five indicators) on three public health dimensions:

- primary diagnostic testing,
-
- national microbiology reference laboratory (NRL) services,
-
- laboratory-based surveillance and epidemic response support.

Read more about EULabCap on the ECDC website.