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The European I-MOVE Multicentre 2013–2014 Case-Control Study. Homogeneous moderate influenza vaccine effectiveness against A(H1N1)pdm09 and heterogenous results by country against A(H3N2)

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Abbreviations: ARI, acute respiratory infection; Df, degrees of freedom; EMA, European Medicines Agency; EU, European Union; HI, hemagglutination inhibition assay; ILI, influenza like illness; I-MOVE, Influenza Monitoring Vaccine Effectiveness in Europe; ISO, International Organization for Standardization; MCCS, multicentre case control study; OR, odds ratio; PCR, polymerase chain reaction; VE, vaccine effectiveness; WHO, World Health Organization; 95% CI, 95% confidence intervals.

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ABSTRACT

Background: In the first five I-MOVE (Influenza Monitoring Vaccine Effectiveness in Europe) influenza seasons vaccine effectiveness (VE) results were relatively homogenous among participating study sites. In 2013–2014, we undertook a multicentre case-control study based on sentinel practitioner surveillance networks in six European Union (EU) countries to measure 2013–2014 influenza VE against medically-attended influenza-like illness (ILI) laboratory-confirmed as influenza. Influenza A(H3N2) and A(H1N1)pdm09 viruses co-circulated during the season.

Methods: Practitioners systematically selected ILI patients to swab within eight days of symptom onset.

We compared cases (ILI positive to influenza A(H3N2) or A(H1N1)pdm09) to influenza negative patients. We calculated VE for the two influenza A subtypes and adjusted for potential confounders. We calculated heterogeneity between sites using the I^2 index and Cochrane's Q test. If the I^2 was <50%, we estimated pooled VE as (1 minus the OR) × 100 using a one-stage model with study site as a fixed effect. If the I^2 was >49% we used a two-stage random effects model.

Results: We included in the A(H1N1)pdm09 analysis 531 cases and 1712 controls and in the A(H3N2) analysis 623 cases and 1920 controls. For A(H1N1)pdm09, the Q test ($p = 0.695$) and the I^2 index (0%) suggested no heterogeneity of adjusted VE between study sites. Using a one-stage model, the overall pooled adjusted VE against influenza A(H1N1)pdm2009 was 47.5% (95% CI: 16.4–67.0).

For A(H3N2), the I^2 was 51.5% ($p = 0.067$). Using a two-stage model for the pooled analysis, the adjusted VE against A(H3N2) was 29.7 (95% CI: −34.4–63.2).

Conclusions: The results suggest a moderate 2013–2014 influenza VE against A(H1N1)pdm09 and a low VE against A(H3N2). The A(H3N2) estimates were heterogeneous among study sites. Larger sample sizes by study site are needed to prevent statistical heterogeneity, decrease variability and allow for two-stage pooled VE for all subgroup analyses.

1. Introduction

In February 2013, the World Health Organization (WHO) recommendations for the 2013–2014 trivalent influenza vaccine for the Northern Hemisphere were to include an A/California/7/2009(H1N1)pdm09-like virus, an A(H3N2) virus antigenically like the cell-propagated prototype virus A/Victoria/361/2011 (A/Texas/50/2012) and a B/Massachusetts/2/2011-like virus. The A/Victoria/361/2011-like vaccine viruses used as A(H3N2) component in the 2012–2013 vaccine had antigenic changes resulting from adaptation to propagation in eggs. Consequently, WHO recommended to use as A(H3N2) 2013–2014 component A/Texas/50/2012 [1]. The A(H1N1)pdm09 vaccine component was the same recommended since 2009.

In Europe, up to 2014, the European Medicines Agency (EMA) required vaccine manufacturers to conduct small clinical trials with the newly recommended seasonal influenza vaccines.

On 23rd January 2014, the EMA notified influenza vaccine producers that from the 2015–2016 season those trials were no longer requested. To strengthen post-marketing monitoring of the performance of influenza vaccines, vaccine manufacturers would be required to provide product-specific vaccine effectiveness results and enhance vaccine safety surveillance [2].

Since 2008–2009, the I-MOVE (Influenza Monitoring Vaccine Effectiveness in Europe) multicentre case control study (MCCS) provides vaccine effectiveness (VE) estimates by influenza type/subtype, age-group, target population and, since 2012–2013 by vaccine type [3–7]. If resources for larger sample size were available, I-MOVE MCCS, could routinely measure influenza VE by product providing results scientifically independent from vaccine manufacturers.

The 2013–2014 influenza season in Europe was mild with co-circulation of A(H1N1)pdm09 and A(H3N2) viruses [8].

In this 6th season of I-MOVE, we aimed to provide 2013–2014 seasonal laboratory confirmed influenza VE against medically-attended influenza like illness (ILI), by influenza A subtype, age-group and vaccine type.

2. Methods

The six study sites participating in the 2013–2014 multicentre case-control study were in Germany, Hungary, Ireland, Portugal, Romania and Spain.

Participating practitioners started to interview ILI patients and carry out naso-pharyngeal swabbing for the study from 15 days after the beginning of the 2013–2014 country-specific seasonal influenza vaccination campaigns. Practitioners selected all older persons (60 or 65 years old and older) and a systematic sample of patients from other age groups consulting with ILI. In Hungary, only patients aged over 18 years were eligible. In Germany in case of no patients consulting for ILI in the week, practitioners sampled those consulting for Acute Respiratory Infection (ARI).

Practitioners collected information on: date of ILI symptom onset and date of swabbing; 2013–2014 seasonal influenza vaccination status, date and brand, 2012–2013 seasonal influenza vaccination status; ILI signs and symptoms, sex, presence of a chronic condition (including obesity, except Germany), number of hospitalisations for chronic conditions in the previous 12 months and pregnancy. Five study sites collected information on the number of practitioner visits in the past 12 months and smoking status (not collected in Germany) and, five on receipt of antivirals (not collected in Spain). Three study sites included a specific question to identify individuals in the target group for influenza vaccination, and three used variables such as age, chronic conditions, pregnancy and professional or care giver status to enable their identification.

We included patients in the study if they met the European Commission ILI case definition (sudden onset of symptoms and at least one of the following four systemic symptoms: fever or feverishness, malaise, headache, myalgia; and at least one of the following three respiratory symptoms: cough, sore throat, shortness of breath), if they were swabbed within seven days of symptom onset , and did not receive antivirals prior to swabbing.

Swabs were tested for influenza using real time polymerase chain reaction (PCR) at the respective country's National Influenza Reference Laboratory. In Spain, tests were also conducted in other laboratories participating in the National Influenza Sentinel

Surveillance System. The corresponding National Influenza Centre genetically characterised a sub-set of positive specimens or isolated viruses by sequencing the HA1 coding portion of the haemagglutinin gene. The Spanish and Portuguese National Influenza Centre analysed all study sequences provided by the laboratories of all study sites and compared the sequences to the reassortant viruses used in most vaccine brands: for A(H3N2) to the reassortant virus X-223A (GISAID number EPI407126), and A(H1N1)pdm09 to the reassortant virus X-179A (GISAID number EPI257201).

We reported amino acid changes if they were present in >5% of the viruses sequenced. We used Neighbour-Joining method and Kimura 2-parameter nucleotide substitution model for phylogenetic analysis. To ensure the comparability of the hemagglutination inhibition assay (HI) between countries, we used the antigenic characterisation results reported by the WHO Collaborating Centre MRC National Institute for Medical Research (Mill Hill, London) [8].

Influenza A(H3N2) and A(H1N1)pdm09 cases were patients with the virus subtype identified and controls were patients testing negative for any influenza virus.

For each study site and for each influenza type/subtype, we defined the start of the study period as the week of onset of the first influenza type/subtype case recruited. The end of the study period was the week of onset of the last influenza type/subtype case after the peak, after which there were at least two consecutive weeks with no further influenza positive cases of this type/subtype.

We defined a patient as vaccinated if he/she had received at least one dose of 2013–2014 seasonal influenza vaccine at least 15 days before ILI symptom onset. We considered all other patients unvaccinated.

For each study site, we computed the odds ratio (OR) of being vaccinated in cases versus controls. We conducted a complete cases analysis excluding patients with missing values for any of the variables We used the Cochran's Q-test and the I^2 index to test the heterogeneity between study sites [9]. In study sites with sample sizes large enough we used adjusted ORs and their standard errors, otherwise we used the crude ORs. If the I^2 was <50%, we estimated the pooled type/subtype influenza vaccine effectiveness (VE) as (1 minus the OR) × 100 using a one-stage model with study site as a fixed effect. If the I^2 was >49% we used a two-stage model using a random effects.

For the 1-stage fixed effect models, we used a logistic regression model to calculate VE including potential confounding factors: age (modelled as a restricted cubic spline with 4 knots), sex, presence of at least one chronic condition (including pregnancy and obesity where available) and week of symptom onset. For country-specific estimates used in the two-stage models, the adjustment variables depended on sample size and included one or more of the following potential confounding factors: age (modelled as restricted cubic spline with 4 knots, or as a binary or categorical variable), sex, presence of at least one chronic condition (including pregnancy and obesity where available) and time of onset (week or month of onset or date of onset modelled as a restricted cubic spline with 4 knots).

We stratified VE into three age groups (0–14, 15–59 and 60 years and above) and into three types of vaccines (adjuvanted, split virion, subunit).

We conducted three sensitivity analyses (1) restricting the study to patients swabbed less than 4 days after symptom onset, (2) restricting to the target population (3) excluding patients vaccinated <15 days after symptom onset.

3. Results

The influenza season in the countries of the six study sites started and peaked at different times, as defined by national thresholds (Table 1). The season started earliest in Portugal, Romania and Spain (week 1, 2014) and latest in Germany, (week 8, 2014). The peak of the influenza season varied from week 4, 2014 in Portugal and Spain to week 11, 2014 in Romania.

Among the 4292 ILI/ARI patients recruited, 3196 ILI patients met the eligibility criteria: 1986 testing negative for all influenza viruses, 34 influenza B, 530 influenza A(H1N1)pdm09, 622 influenza A(H3N2), one A(H1N1)pdm09/A(H3N2) co-infection and 22 influenza A not subtyped. Due to the small number of influenza B cases, we restricted the analysis to influenza A(H1N1)pdm09 and A(H3N2). After excluding patients with symptom onset outside the defined study period, we included in the A(H1N1)pdm09 analysis 531 cases and 1712 controls and in the A(H3N2) analysis 623 cases and 1920 controls (the case of co-infection was included in both analyses) (Fig. 1). The maximum weekly number of cases recruited occurred in week 2, 2014 for A(H3N2) and in week 4, 2014 for A(H1N1)pdm09 (Fig. 2).

Among the 3020 included individuals with information on vaccination status, 327 (10.8%) were vaccinated. The proportion of vaccinated varied by study site from 7.6% in Romania (11 vaccinated) to 18.4% in Portugal (23 vaccinated).

Two hundred eighty three (86.5%) of the vaccinated individuals had information on the vaccine brand received; eight different brands were used. By vaccine type, 63% had received inactivated split vaccine, 19% inactivated subunit vaccine and 17% adjuvanted vaccine.

The proportion vaccinated with the 2013–2014 influenza vaccine was 11.8% among controls, 6.5% among A(H1N1)pdm09 cases and 11.7% among A(H3N2) cases (Table 2).

The median age was lower in controls (24 years) compared to A(H3N2) cases (33 years) and A(H1N1)pdm09 cases (35 years) (Table 2). 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 A(H3N2), A(H1N1)pdm09 cases and controls. Eighty-six percent of controls were swabbed within three days of symptom onset compared to 91.7%, and 90.5% of influenza A(H1N1)pdm09 and A(H3N2) cases, respectively.

After excluding patients with missing information on 2013–2014 seasonal vaccination status or date, onset date, age, sex or presence of chronic condition, we included 2113 for the complete case analysis of VE against influenza A(H1N1)pdm09 and 2369 for the analysis against A(H3N2).

The Q test and I^2 index testing for heterogeneity of adjusted VE between study sites suggested no statistical heterogeneity for influenza A(H1N1)pdm09 ($p = 0.695$, $I^2 = 0\%$) and therefore we used a one-stage model to estimate VE against A(H1N1)pdm09.

The overall VE against influenza A(H1N1)pdm2009 adjusted for onset week, chronic conditions, age and sex, was 47.5% (95% CI: 16.4–67.0) (Table 3).

The adjusted VE was 64.4% (95% CI: −85.8–93.2) among the 0–14 years old, 38.8% (95% CI: −14.2–67.2) among the 15–59 years old. The crude VE was 51.8% (95% CI: −05–76.9) among the 60 years and more. Due to the small sample size in this group, adjustment was not possible.

By vaccine type, the point estimate adjusted VE against A(H1N1)pdm09 were higher for adjuvanted vaccines (72.6%; 95% CI −22.4–93.9) than for inactivated subunit (36.8%; 95% CI −83.3–78.2)) or split virion (44.0%; 95% CI 5.0–65.0)) (Table 3).

In the sensitivity analyses, the overall adjusted VE against A(H1N1)pdm09 was 46.7% (95% CI: 12.4–67.6) when restricting to patients swabbed less than four days after onset of symptoms, 51.5% (95%CI: 14.2–72.6) when restricting to the target group for vaccination, and 47% (95% CI 15.5–66.7) when excluding patients vaccinated less than 15 days before symptom onset (Table 3).

Table 1
Study details; Influenza vaccine effectiveness multicentre case-control study, study sites in six European Union countries, week 47/2013–week 19/2014.

Study site	Week of start of influenza season[a]	Week of peak of influenza season[a]	Number of practitioners recruiting at least one ILI patient[b]	Number of ILI patients[b] included in study	Inclusion period for the final analysis (ISO weeks)[c]	Number of included ILI patients positive to influenza and with known vaccination status[d]		Number of included ILI patients negative for any influenza and with known vaccination status[d]	
Germany	Week 8/2014	Week 10/2014	99	1291	Week 47/2013–week 19/2014	178	18	992	98
Hungary	Week 6/2014	Week 8/2014	47	341	Week 3/2014–week 14/2014	83	6	258	40
Ireland	Week 6/2014	Week 9/2014	21	173	Week 51/2013–week 17/2014	103	8	70	14
Portugal	Week 1/2014	Week 4/2014	24	125	Week 50/2013–week 8/2014	77	9	48	14
Romania	Week 1/2014	Week 11/2014	58	147	Week 1/2014–week 15/2014	75	3	72	8
Spain	Week 1/2014	Week 4/2014	186	1119	Week 49/2013–week 15/2014	680	66	439	48
Total			435	3196		1196	110	1879	222

ILI: Influenza-like illness. ISO: International Organization for Standardization.

[a] According to the thresholds used to define the start of the influenza season in each of the countries.

[b] ILI patients meeting the European Commission case definition, swabbed less than eight days after onset of symptoms within the study period.

[c] From 15 days after the start of the vaccination campaign to week 19, 2014. We excluded controls with onset of symptoms in the weeks before the week of the first influenza case and after the week of the last influenza case after which there were two or more consecutive weeks of no cases in the study site.

[d] ILI patients included in the study, after excluding those with missing information on vaccination status or date of vaccination.

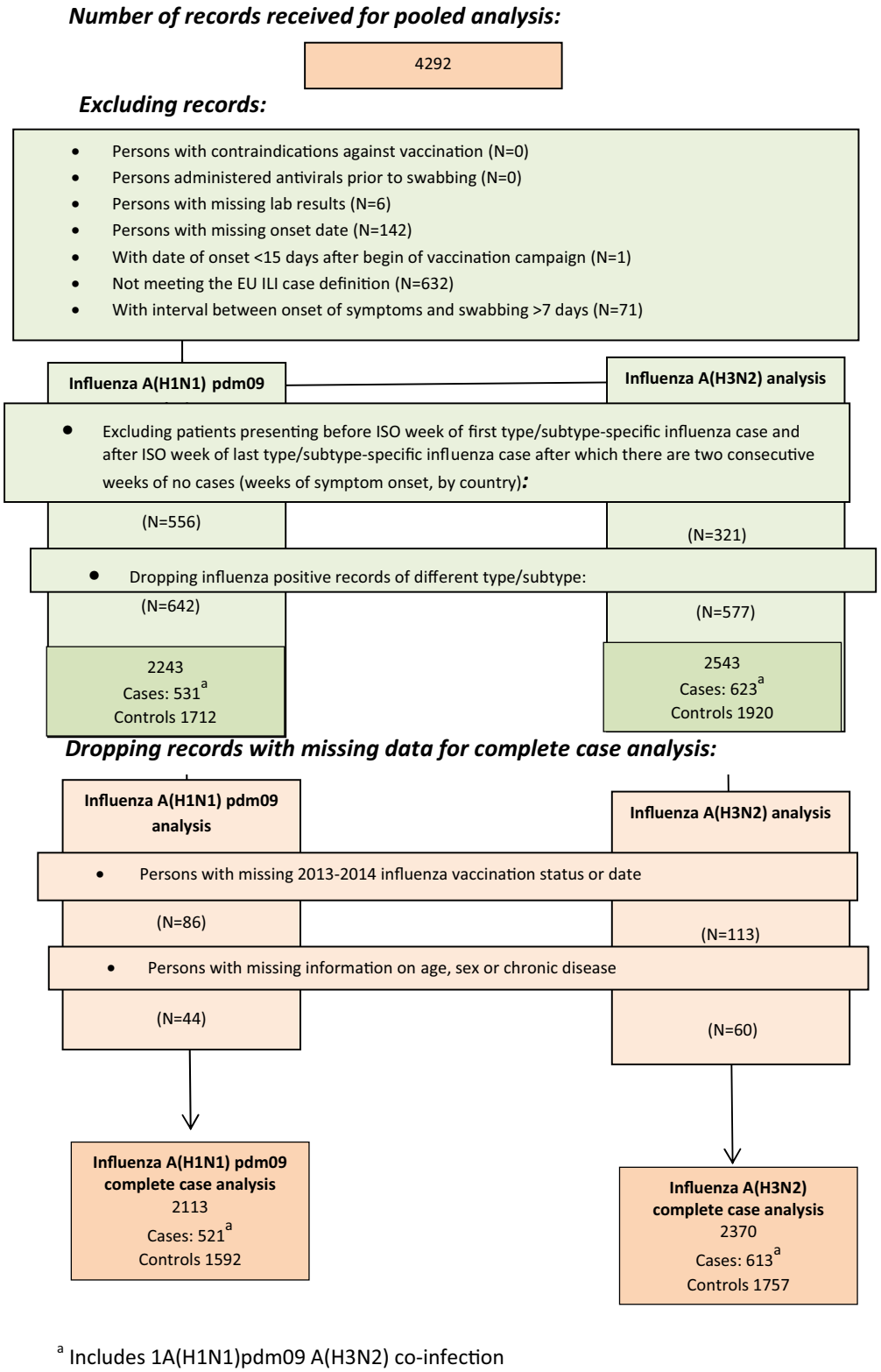

Fig. 1. Flowchart of data exclusion for pooled analysis, I-MOVE multi-centre case-control study, influenza season 2013–2014.

For A(H3N2), the I^2 was 51.5% ($p = 0.067$), and therefore, we used a two-stage model for the pooled VE analysis against A(H3N2). The crude and adjusted VE varied by study site (Table 4). The overall adjusted VE was 30.2% (95% CI: −33.9–63.6). The adjusted two-stage pooled VE against A(H3N2) was not calculated by age group or vaccine group as adjustment was not possible due to sparse data by country.

Eighty-two of the 531 A(H1N1)pdm09 viruses (15:4%) were sequenced (Germany 5, Ireland 9, Portugal 12, Romania 3 and Spain 53). All clustered into the group 6, characterised by D97N and

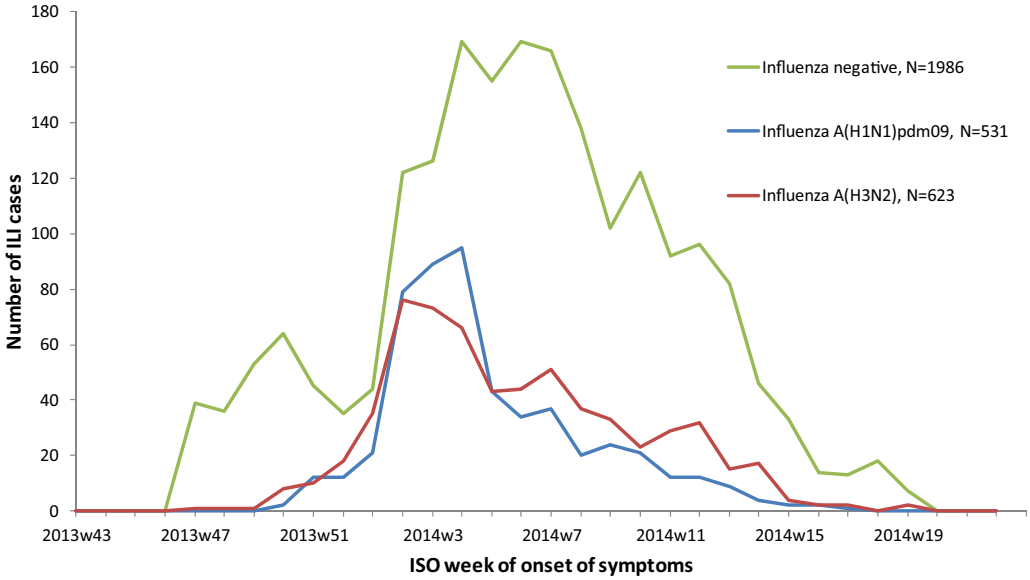

Fig. 2. Number of influenza A(H1N1)pdm09, influenza A(H3N2) and influenza negative ILI cases included in the study by week of symptom onset, pooled I-MOVE dataset, influenza season 2013–2014.

S185T substitutions, subgroup B, carrying additional substitutions: K163Q, K283E and A256T. The mutations K209T and R223Q were present in all the 82 viruses and may be explained by egg adaptation. Other mutations present in all viruses were P83S, S203T. In addition, the mutation I321V was present in 54 of the 73 sequences that included this portion of the haemaglutinine gene (9 sequences from Ireland did not cover it). A total of 54/73 viruses harbored in addition the mutation I321V. All seven viruses analysed by HI assay were antigenically similar to the vaccine virus, A/California/7/2009 (less than 2-fold reduction in HI titre).

Of the 623 A(H3N2) viruses from patients included in the analysis 102 (16.4%) were genetically characterised (Germany 21,

Table 2
Details for influenza, A(H3N2) ($n = 623$)[a], A(H1N1)pdm09 ($n = 531$)[a] cases and controls[a] ($n = 1986$) included in the 2013–2014 season trivalent influenza vaccine effectiveness analysis, multicentre case control study in six European Union study sites, week 50 (2013)–week 17 (2014), influenza season 2013–2014.

Variables	Number of test-negative controls [b]/total *n*(%)	Number of influenza A(H1N1)pdm09[b]/total *n*(%)	Number of influenza A(H3N2) cases[b]/total *n*(%)
Median age (Interquartile range), years	24.0 (4–45)	35.0 (18–47)	33.0 (12–48)
Age groups (years)			
0–4	498/1983 (25.1)	35/531 (6.6)	70/623 (11.2)
5–14	317/1983 (16.0)	80/531 (15.1)	109/623 (17.5)
15–64	969/1983 (48.9)	374/531 (70.4)	366/623 (58.7)
≥60	199/1983 (10.4)	42/531 (7.9)	78/623 (12.5)
Sex			
Female	1031/1976 (52.2)	262/531 (49.3)	308/621 (49.6)
Days between onset of symptoms and swabbing			
0	142/1986 (7.2)	18/531 (3.4)	32/623 (5.1)
1	723/1986 (36.4)	214/531 (40.3)	230/623 (36.9)
2	522/1986 (26.3)	168/531 (31.6)	193/623 (31.0)
3	318/1986 (16.0)	87/531 (16.4)	109/623 (17.5)
4–7	281/1986 (14.0)	44/531 (8.4)	59/623 (9.0)
Seasonal vaccination, 2013–2014[c]	222/1879 (11.8)	34/523 (6.5)	72/617 (11.7)
2013–2014 influenza vaccine type			
Inactivated subunit	37/1843 (1.9)	5/521 (0.9)	13/611 (2.1)
Inactivated split virion	105/1843 (5.3)	25/521 (4.7)	49/611 (7.9)
Adjuvanted	43/1843 (2.2)	2/521 (0.4)	4/611 (0.6)
At least one chronic condition	389/1920 (20.3)	102/529 (19.3)	128/616 (20.8)
At least one hospitalisation in the previous 12 months for chronic conditions	32/1964 (1.6)	6/529 (1.1)	10/620 (1.6)
Belongs to target group for vaccination	499/1953 (25.6)	131/531 (24.7)	163/619 (26.3)
Study sites			
Germany	1099/1986 (55.3)	57/531 (10.7)	117/623 (18.8)
Hungary	258/1986 (13.0)	41/531 (7.7)	26/623 (4.2)
Ireland	70/1986 (3.5)	41/531 (7.1)	54/623(8.7)
Portugal	48/1986 (2.4)	49/531 (9.2)	28/623 (4.5)
Romania	72/1986 (3.6)	19/531 (3.6)	52/623 (8.3)
Spain	439/1986 (22.1)	324/531 (61.0)	346/623(55.5)

[a] One influenza cases positive for both influenza A(H1N1)pdm09 and A(H3N2) was included in the analysis.
[b] Controls from "any influenza" analysis used.
[c] Vaccination more than 14 days before onset of influenza like illness symptoms.

Table 3
Pooled one-stage model crude and adjusted seasonal vaccine effectiveness against laboratory confirmed influenza A(H1N1)pdm09 overall, by age group and by vaccine type. Multicentre case-control study in six European Union study sites, week 50 (2013)–week 17 (2014), influenza season 2013–2014.

	Analysis scenarios, population included		A(H1N1)pdm09 VE (%)	(95%CI)
Primary analysis	All age groups[a]	*N* (cases/vaccinated; controls/vaccinated)	2113 (521/34; 1592/203)	
		Crude	52.2	(30.3–67.3)
		Crude (study site as fixed effect)	57.4	(36.3–71.5)
		Adjusted for sex	57.6	(36.5–68.7)
		Adjusted for chronic condition	55.6	(34.9–67.5)
		Adjusted for age (cubic spline)	49.1	(20.5– 67.5)
		Adjusted for onset week, age (cubic spline)	48.5	(18.7–67.3)
		Adjusted for onset week, chronic condition	54.5	(30.2–70.4)
		Adjusted for study site, onset week, age (cubic spline), chronic conditions, sex	47.5	(16.4–67.0)
	0–14 years old	*N* (cases/vaccinated; controls/vaccinated)	698 (110/2; 588/46)	
		Crude	78.2	(8.8–94.8)
		Crude (study site included as fixed effect)	75.6	(−17.0–94.9)
		Adjusted for study site, onset month, age (cubic spline),chronic condition[a]	64.4	(−85.8–93.2)
	15–59 years old[b]	*N* (cases/vaccinated; controls/vaccinated)	1189 (369/17; 820/61)	
		Crude	39.9	(−4.4–65.4)
		Crude (study site included as fixed effect)	37.1	(−12.7–64.9)
		Adjusted for study site, onset week, age (cubic spline), chronic condition, sex[c]	38.8	(−14.2–67.2)
	60 years old and above[d]	*N* (cases/vaccinated; controls/vaccinated)	226 (42/15; 184/96)	
		Crude	49.1	(−2.0–74.9)
		Crude (study site included as fixed effect)	51.8	(−0.5–76.9)
		Adjusted	Too few cases	
	Analysis by vaccine type	*N* (cases/vaccinated subunit/vaccinated split virion/vaccinated adjuvanted; controls/vaccinated subunit/vaccinated split virion/vaccinated adjuvanted)	2113 (521/5/25/2/; 1592/32/99/43)	
		Crude[e] subunit	51.4	(−34.1–82.4)
		Crude[e] split virion	53.7	(25.3–71.3)
		Crude[e] adjuvanted	79.2	(10.7–95.1)
		Adjusted[f] subunit	36.8	(−83.3–78.2)
		Adjusted[f] split virion	44.0	(5.0–65.0)
		Adjusted[f] adjuvanted	72.6	(−22.4–93.9)
Sensitivity analysis	Restricted to target group for vaccination[g]	*N* (cases/vaccinated; controls/vaccinated)	528 (130/26; 398/144)	
		Crude	55.9	(29.0–72.6)
		Crude (study site included as fixed effect)	60.6	(34.9–76.2)
		Adjusted for study site, onset week, age (cubic spline), chronic condition, sex	51.5	(14.2–72.6)
	Restricted to those with delay onset and swabbing < 4 days	*N* (cases/vaccinated; controls/vaccinated)	1846 (477/30; 1369/163)	
		Crude	50.3	(25.6–66.9)
		Crude (study site included as fixed effect)	58.0	(35.4–72.7)
		Adjusted for study site, onset week, age (cubic spline), chronic condition, sex	46.7	(12.4–67.6)
	Individuals vaccinated < 15 days excluded	*N* (cases/vaccinated; controls/vaccinated)	2108 (519/34; 1589/203)	
		Crude	52.1	(30.2–67.2)
		Crude (study site included as fixed effect)	57.3	(36.1–71.4)
		Adjusted for study site, onset week, age (cubic spline), chronic condition, sex	47.0	(15.5–66.7)

[a] Portugal excluded (four cases unvaccinated).
[b] Weeks of inclusion: week 50 (2013)–week 17(2014).
[c] Week 16 excluded (one record dropped).
[d] Weeks of inclusion: week 52(2013)–week 13 (2014).
[e] S**t**udy site included as fixed effect.
[f] Adjusted for study site, onset week, age (cubic spline), chronic condition, sex.
[g] Weeks of inclusion: week 50 (2013) week 13 (2014).

Table 4
One-stage and two-stage pooled crude and adjusted seasonal vaccine effectiveness against laboratory confirmed influenza A(H3N2) overall and by age group. Multicentre case control study in six European Union study sites, week 47 (2013)–week 19 (2014), influenza season 2013–2014.

	Study site	*N* (cases/vaccinated; controls/vaccinated)	VE (%)	95% CI
All ages crude estimates	Germany	1045 (107/15; 938/94)	−46.4	(−163.0 18.5)
	Hungary	223 (26/1; 197/33)	80.1	(−51.9–97.4)
	Ireland	122 (54/5; 68/14)	60.6	(−17.3–86.8)
	Portugal	75 (28/6; 47/14)	35.7	(−92.7–78.6)
	Romania	124 (52/1; 72/8)	84.3	(−29.5–98.1)
	Spain	781 (346; 44/435/48)	−17.5	(−81.6–24.0)
	2-stage crude pooled estimate	2370 (613,72/1757,211)	24.6	(−33.4–57.4)
	Heterogeneity chi-squared = 10.95 (df = 5), $p = 0.052$ *I*-squared = 54.4%			
All ages adjusted estimates	Germany	1045	−36.4	(−160.0–28.5)
	Adjusted for onset week (cubic spline), chronic condition, age (cubic spline), sex			
	Hungary	223	91.6	(26.4–99.0)
	Adjusted for age group (two categories 18–59, 60+)			
	Ireland	122	60.7	(−41.4–89.1)
	Adjusted for onset month, chronic condition, age group (three categories: 0–14, 15–59, 60+)			
	Portugal	75	23.0	(−209–80.9)
	Adjusted for onset month, chronic condition, age group (two categories: 0–59, 60+)			
	Romania	124	82.7	(−66.0–98.2)
	Adjusted for onset month, chronic condition, age group (three categories: 0–14, 15–59, 60+)			
	Spain	781	−12.2	(−95.7–35.7)
	Adjusted for onset week (cubic spline), chronic condition, age (cubic spline), sex			
	2-stage adjusted pooled estimate	2370 (613,72/1757,211)	30.2	(−33.9–63.6)
	Heterogeneity chi-squared = 10.43 (d.f. = 5), $p = 0.064$ *I*-squared = 52%			
15–59 years crude estimates	Germany	347 (37/3; 310/26)	3.6	(−235.4–73.3)
	Hungary	181 (19/0; 162/11)	NA	NA
	Ireland	82 (34/2; 48/6)	56.3	(−131.3–91.7)
	Portugal	49 (15/2; 34/5)	10.8	(−421.5–84.7)
	Romania	69 (30/1; 39/3)	58.6	(−319.2–95.9)
	Spain	497 (228/14; 269/14)	−19.2	(−155.5–44.4)
	2-stage crude pooled estimate (Hungary excluded)	1044 (76/22; 968/322)	6.6	(−62.9–46.5)
	Heterogeneity chi-squared = 1.67, (df = 4) $p = 0.796$ *I*-squared = 0.0 = %			
60 years and over crude estimates[a]	Germany	64 (7/2; 57/24)	45.0	(−207.8–90.2)
	Hungary	42 (7/1; 35/22)	90.2	(8.8–98.9)
	Ireland	17 (4/2; 13/7)	NA	NA
	Portugal	23 (10/4; 13/9)	70.4	(−66.9–94.7)
	Romania	16 (5/0; 11/5)	NA	NA
	Spain	102 (45/29; 57/28)	−87.7	(−318.4–15.8)
	2-stage crude pooled estimate	231 (69/36; 162/83)	47.4	(−97.5–86.0)
	Heterogeneity chi-squared = 8.89 (df 3) $p = 0.031$ *I*-squared = 66.3%			

[a] Weeks of inclusion 50 (2013)–14 (2014); VE: vaccine effectiveness; CI: confidence intervals; NA: not applicable; df: degrees of freedom.

Hungary 1, Ireland 8, Portugal 4, Romania 4 and Spain 64) (Table 5). All clustered into the group 3C corresponding to the recommended vaccine virus for the 2013–14 seasons A/Texas/50/2012.

We identified 12 amino acid position changes. Three of these (V186G, F219S and N226I) were present in the 102 analysed viruses and could be explained by egg adaptation. Four mutations (N128T/A, R142G, N145S and P198S) were already present in A(H3N2) viruses circulating in 2012–2013. Sixty three viruses (62%) harbored mutations detected for the first time in the 2013–2014 season. All 63 presented the L157S mutation located in the B antigenic site close to the receptor binding site; 51 of them had a second mutation located in the A antigenic (N122D) and six of these three additional mutations located in the E antigenic site (E62K, K83R and R261Q) were in six of these viruses (Table 5).

Among the 12 A(H3N2) viruses analysed by HI, nine showed an eightfold or higher reduction in HI antibody titers when using post-infection ferret antiserum raised against the egg-propagated A/Texas/50/2012 vaccine virus. The 12 viruses presented ≤4-fold

Table 5
Number of strains with amino acid changes in the Haemagglutinin genes A(H3N2) relative to vaccine reference strain by genetic subgroup and study site. I-MOVE multicentre cases-control study influenza, influenza season 2013–2014.

Study site	Subgroup 3 C.2	Subgroup 3 C.3			
	N128T	N128A, R142G	L157S	L157S; N122D	L157S; N122D; E62K; K83R; R261Q
Germany	5	10	5	1	
Hungary	1				
Ireland		1		1	6
Portugal	2	1	1		
Romania		1	2	1	
Spain	6	12	4	42	
Total	14	25	12	45	6

reduction when using ferret antisera raised against other viruses similar to A/Texas/50/2012 propagated in cells.

4. Discussion

The results of the I-MOVE multicentre case control suggest a moderate 2013–2014 influenza VE against A(H1N1)pdm09 and a low VE against A(H3N2).

All the overall adjusted estimates for A(H1N1)pdm09 including those calculated in the sensitivity analysis ranged between 46.7% and 51.5%. They were similar to our estimates in 2012–2013 (50.4%) and 2011–2012 (56.1%) [5,6]. The highest point VE was among children 0–14 years and the lowest among those aged 60 years and over. However, the low proportion of children vaccinated (8.2% vaccinated among controls) and the small sample size among patients aged 60 years and over resulted in imprecise estimates. The laboratory results indicate that the strains isolated from study participants were similar to that included in the vaccine.

Among those vaccinated, the completeness of vaccination status was good (87%). The point estimate VE was higher for adjuvanted vaccines but the small sample size resulted in imprecise estimates. With the large number of influenza vaccines used in Europe and the low vaccination coverage, a much larger sample size is needed to provide VE by product as requested by EMA [2].

The VE against A(H3N2) was similar to the estimates from the 2011–2012 I-MOVE MCC (24.8%) [4]. In February 2014 Spain and Navarra region, sites included in the MCCS reported early estimates of 28% and 13% against A(H3N2) respectively [10,11]. In Navarra, and Ireland A(H3N2) outbreaks in highly vaccinated nursing homes occurred in the 2013–2014 winter suggesting poor performance of the vaccine [10,12]. The low VE observed could be explained by the amino acid mutations located in the B antigenic site, close to the receptor binding site. Changes in close amino acid positions in the B antigenic site (155, 156, 158 and 159) have determined major antigenic changes during the influenza virus evolution [13]. However, the results indicate that the viruses from patients included in our study were antigenically similar to the 2013–2014 vaccine virus.

For the first time since 2008–2009, the estimates for A(H3N2) were heterogeneous among study sites. To account for this heterogeneity, we used a two-stage model to provide pooled adjusted estimates. Two-stage models give more weight to small study sites than a one-stage model, if there is heterogeneity [14]. In a two-stage model, the sample size required by study site is higher as we pool study site adjusted VE. Because of sparse data, we could only provide overall adjusted VE.

Several factors can explain the heterogeneity among study sites. First, a different application of the study protocol may result in heterogeneity. However, all sites had already participated in previous I-MOVE MCCS and there were no changes in the study procedures at study site level compared to previous seasons. Moreover, if there were differences in the application of the protocol we would also expect heterogeneity between study sites for the A(H1N1)pdm09 VE estimates. Second, heterogeneity may be due to difference in the viruses circulating in the participating study sites or to different vaccines being used. The limited number of viruses characterised by study site and the small number of individuals vaccinated with each of the vaccines did not allow the verification of this hypothesis. Third, we cannot exclude the presence of information or selection bias. However, if bias was present, we would also expect high heterogeneity for A(H1N1)pdm09 VE estimates. Fourth, the low vaccine coverage in controls and the small sample sizes for stratification resulted in a very high variability of the estimates. In any case, if presence of statistical heterogeneity is identified in the future, larger sample sizes by study site should be achieved to provide two-stage pooled VE for all subgroup analysis and for better interpretation of the results.

VE against A(H1N1)pdm09 was moderate and in the range of what we have observed since the pandemic. The VE against A(H3N2) was lower with heterogeneous estimates among study sites. As in 2012–2013 the high completeness of the information on the vaccine product allowed having estimates by vaccine type but the low vaccination coverage impeded having estimates by product. The I-MOVE MCCS is a strong network of scientists who through robust methods can provide information needed to guide influenza vaccination programmes in the EU/EEA. However, in order to provide precise EU estimates by age group and vaccine-product and to account for potential heterogeneity, a larger sample size and a higher number of study sites are needed. In addition to better integrate the laboratory results in the interpretation of influenza VE estimates, the number and representativeness of virus strains characterised should be increased.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2015.04.012

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